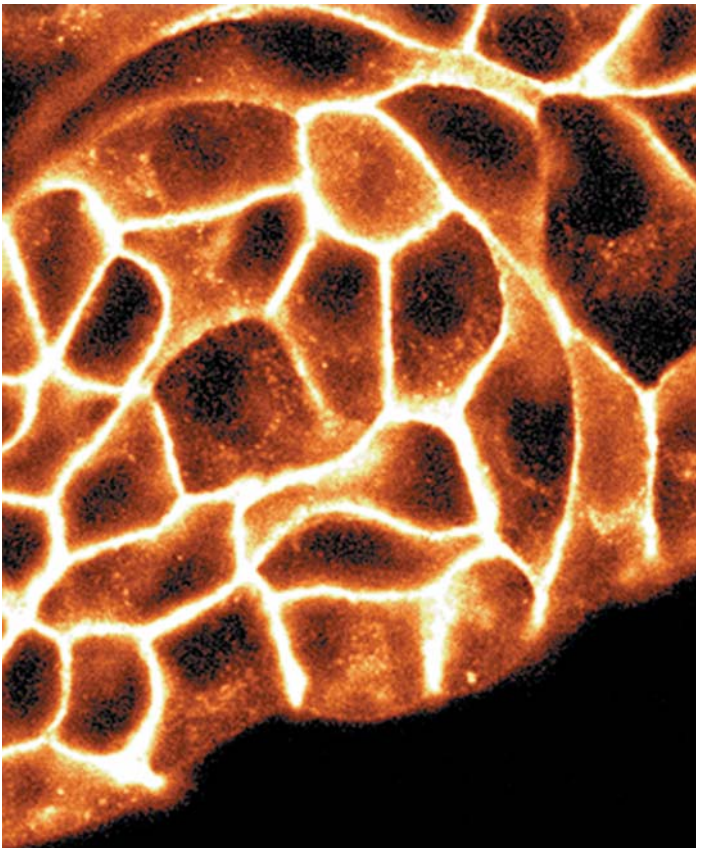
BD Biosciences

Cell Adhesion Protocol Manual



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Cell Adhesion

1ST Edition September 2001

Protocol Manual

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Introduction

Adhesion molecules enable cells to contact and specifically interact with each other, a requirement of all multicellular organisms. Specialized cell-cell interactions allow communication between adjacent cells and are crucial for cell development and functional activity. In addition to contacting neighboring cells, cells interact with the extracellular matrix (ECM), a complex assortment of proteins and carbohydrates found in spaces between cells. It lies beneath epithelial and endothelial layers, and surrounds all connective tissue cells. The ECM provides strength and physical support in tissues and plays an important role in regulating cellular activities.

Several different families of receptors mediate cell-cell and cell-matrix interactions. The families of adhesion molecules identified to date include selectins, integrins, cadherins, and Ig superfamily members. Members of these adhesion receptor families play critical roles in embryonic development, differentiation, migration, inflammation, cancer metastasis, and wound healing. The mechanisms regulating adhesive interactions are complex. A single cell can express an array of different adhesion receptors, and a single receptor may bind to more than one ligand. Both expression and functional activity of adhesion molecules are influenced by a variety of factors, including the developmental stage of the cell and the presence of activating factors such as specific antigen and inflammatory mediators. In addition, different adhesion receptors may operate successively over time. This has been well demonstrated for leukocyte extravasation into sites of inflammation, in which adhesion molecules from the selectin, integrin, and immunoglobulin receptor families work in succession as part of an "adhesion cascade".

Due to the complexity of cell adhesion, the role of a particular molecule may be best studied using a multiparameter approach. Specific mRNAs may be detected by a multi-probe ribonuclease protection assay such as BD RiboQuant™ (**Section III E**). Adhesion molecules can be analyzed at the protein level using a variety of techniques including immunofluorescence and flow cytometry (**Section III A**), immunohistochemistry (**Section III B**), ELISA, and immunoblotting (**Section III D**). Cell based assays allow for the quantitation of functional activity (**Section IV**). The role of adhesion molecules can also be assessed by specifically blocking adhesion either *in vitro* or *in vivo*. Finally, the use of transgenic mice or mice with targeted deletions of adhesion molecules allows further study of the physiological role of a specific adhesion molecule.

In recent years there has been a great deal of progress in the field of cell adhesion. New adhesion molecules have been identified, and our knowledge of the characteristics and regulation of molecules expressed by different cell types has been expanded. The role of adhesion molecules in transmitting activation signals to cells has been identified, and the specific mechanisms involved are under investigation. These advances have highlighted the importance of cell adhesion in the pathology of many diseases, including many bacterial and viral infections, acute and chronic inflammation, cancer, and cardiovascular disease. Continued research in this area will aid in the effort to identify potential therapeutic targets.

This Cell Adhesion Protocol Manual is intended to provide investigators with an integrated set of methods for studying cell adhesion. Each section provides a detailed protocol featuring a variety of new reagents for investigation of cell adhesion. For each protocol, suggested controls and helpful hints are included. This information is intended to benefit both the experienced researcher and those new to the field of cell adhesion.

Techniques

I. Cell Culture

The extracellular matrix (ECM) is a vital component of the cellular microenvironment *in vivo*. It serves as the framework for cell anchorage and plays a dynamic role in the regulation of cell growth and function. Although the ECM of different tissues is specialized, the components commonly include collagens (Col), fibronectin (FN), fibrinogen (Fg), laminins (LM), proteoglycans, and vitronectin (VN). Purified ECM proteins can be used in *in vitro* culture systems to provide an appropriate environment for specific cell types and applications. The presence of ECM components promotes cell attachment, spreading, growth and differentiation of a variety of primary cells and cell lines. For some cells the presence of particular ECM components is critical for optimum cell growth *in vitro*. (**Figure 1.** pg. 3). Purified ECM proteins (**Table I.** pg. 3) can be easily used to coat the desired tissue culture surface. Alternatively, BD BioCoat™ Cellware, including tissue culture plates, dishes, flasks, coverslips, and culture slides are available pre-coated with a variety of ECM proteins and attachment factors (**Table II.** pg. 4).

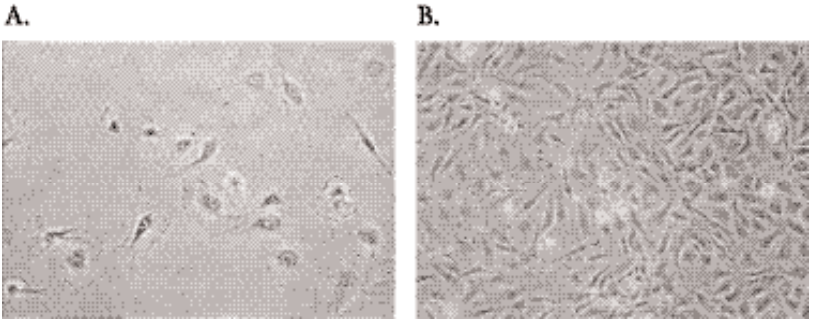


Figure 1. Effects of BD BioCoat™ Endothelial Cell Growth Environment on human umbilical vein endothelial cells (HUVEC). HUVECs grown for 5 days in medium containing 10% FBS on tissue-culture plastic show sparse growth (A) while HUVECs grown using BD BioCoat™ Endothelial Cell Growth Environment form a confluent monolayer and show numerous mitotic cells (B).

II. Cell Culture Product List

Table I. Extracellular Matrix Proteins

Reagent	Quantity	Cat. No.
BD Matrigel™ Matrix	5 ml	356234
	10 ml	354234
	5 × 10 ml	356235
BD Matrigel™ Matrix Phenol-red free	10 ml	356237
GFR BD Matrigel™ Matrix	5 ml	356230
	10 ml	354230
GFR BD Matrigel™ Matrix Phenol-red free	10 ml	356231
Collagen I, bovine	30 mg	354231
Collagen I, human	0.25 mg	354243
Collagen I, rat tail	100 mg	354236
	1 g	356236
Collagen III, human	0.25 mg	354244
Collagen IV, human	0.25 mg	354245
Collagen IV, mouse	1 mg	354233
	10 mg	356233
Collagen V, human	0.25 mg	354246
Poly-D-Lysine	20 mg	354210
Fibronectin, human	1 mg	354008
	5 mg	356008
	25 mg	356009
Laminin, mouse	1 mg	354232
Ultrapure Laminin, mouse	1 mg	354239
Vitronectin, human	0.25 mg	354238

Table II. BD BioCoat™ Cellware

		Col I	Col IV	FN	LM	BD Matrigel™ Matrix
Multiwell plates	6-well	356400	354428	354402	354404	354432
	12-well	356500		354501	354502	354503
	24-well	356408	354430	354411	354412	354433
	48-well	356505		354506	354507	354508
	96-well	356407	354429	354409	354410	
	384-well	356666				
Culture dishes	35 mm	356456	354459	354457	354458	354460
	60 mm	356401	354416	354403	354405	
	100 mm	356450	354453	354451	354452	
	150 mm	354551	354554	354552	354553	
Flasks	T-25	356451	354534	354532	354533	
	T-75	356462	354523	354521	354522	
	T-150	356645		354646		
	T-175	356478	354528	354526	354527	
Coverslips	12 mm round					
	22 mm round	354089		354088		
Culture Slides	1-well	354556		354558		
	2 -well	354627		354628		
	4-well	354557		354559		
	8 -well	354630		354631		

Table II. BD BioCoat™ Cellware *(continued)*

		BD Matrigel™ Matrix Thin layer	PDL	PLL	PDL/ Laminin	PLO/ Laminin	EHS Natrix
Multiwell plates (*cont.*)	6-well	354603	356413	356515	354595	354418	
	12-well		356470				
	24-well	354605	356414		354619		354419
	48-well		356509				
	96-well	354607	356461	356516	354596		
	384-well		356662				
Culture dishes (*cont.*)	35 mm	354602	356467	356518			
	60 mm	354601	356468	356517			
	100 mm	354600	356469		354455		
	150 mm		354550				
Flasks (*cont.*)	T-25		356479				
	T-75		356524				
	T-150		356538				
	T-175		356529				
Coverslips (*cont.*)	12 mm round		354086	354085	354087		
	22 mm round						
Culture Slides (*cont.*)	1-well		354566				
	2 -well		354629		354687		
	4-well		354577				
	8 -well		354632		354688		

*Please see the BD Biosciences Discovery Labware Catalog for a complete product listing.

III. Analysis of Cell Adhesion Molecule Expression

A. Flow Cytometric Analysis

Flow cytometric analysis is a powerful analytical technique in which individual cells can be analyzed for several parameters simultaneously. Light scatter signals can distinguish cell subpopulations based on structural and morphological features while fluorescent antibodies identify specific cell molecules (**Figure 2**). Flow cytometric analysis is an essential tool in many fields of study, including analysis of cell adhesion molecule expression. A general protocol for immunofluorescent staining of cell surface adhesion molecules is outlined here.

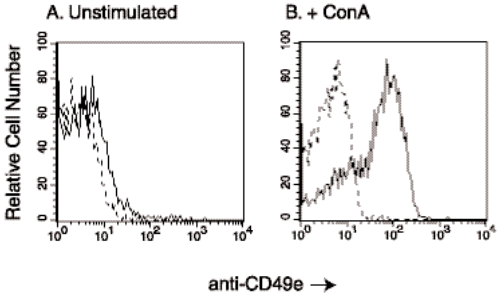


Figure 2. Expression of CD49e (α5 integrin) is upregulated on activated cells. Mouse splenocytes were stained with either anti-mouse CD49e-PE (clone 5H10-27) (solid lines) or an isotype control (clone R35-95) (dashed lines). Cells were either unstimulated (A) or activated by culture with Con A (B). Splenocytes were analyzed by multiparameter flow cytometry.

Protocol

1. Cell Preparation

a) Prepare a single cell suspension from cultured cells (See note #1) or freshly harvested tissue. Red blood cells from peripheral blood or a mouse spleen cell suspension can be removed by density gradient centrifugation (See note #2) or by lysis (See note #3).

b) Resuspend cells in cold wash buffer (PBS with 0.1% NaN3 and 1.0% fetal bovine serum, pH 7.2 - 7.4) at a concentration of 2×10^7 cells/ml. Aliquot 0.05 ml of cell suspension (10^6 cells) into tubes or microwell plates for staining.

2. Staining

a) Add primary mAbs (*e.g.*, unconjugated, biotinylated, or fluorochrome-conjugated mAbs) to deliver predetermined optimal concentration. (See note #4.) Mix gently and incubate at 4°C for 20 - 40 min in the dark.

b) To wash cells, add 0.2 ml of wash buffer per microwell or 1 ml per 4 ml tube and centrifuge at 350 × *g* for 5 min. Wash two times. If a biotin-conjugated primary antibody is used, three washes are recommended.

c) If a second-step reagent is needed for detection, resuspend cell pellet in 0.1 ml of wash buffer with an optimum concentration of secondary reagent. Incubate at 4°C for 20 - 40 min in the dark.

d) Wash two times with 0.2 ml wash buffer as in Step 2b.

e) Resuspend cells in 0.5 - 1.0 ml of wash buffer in tubes appropriate for flow cytometer. Unfixed samples should be analyzed as soon as possible. Fixed samples may be stored at 4°C, covered (See note #5).

Controls

Every experiment must include controls. Negative controls are samples of the same cell population treated exactly as the test sample, but with the omission or modification of one of the staining steps. Examples of negative controls are unstained cells, cells incubated with the 2nd step reagent alone, or with an Ig isotype control which is the same isotype and format (*e.g.*, purified, biotin or fluorochrome) as the primary antibody. For multi-color staining, single-color stained controls must also be included for setting fluorescent signal compensation. If possible, when analyzing markers on an uncharacterized cell type, cells which are known to express the antigen of interest should be included in each experiment as a positive control.

Notes

#1 To prepare a single cell suspension from adherent cell lines, addition of trypsin and/or EDTA may be required. Please note that some cell surface antigens are sensitive to trypsinization. Also, EDTA should be washed away prior to staining.

#2 For enrichment of lymphocytes and removal of red blood cells by density gradient centrifugation, reagents such as Lympholyte (CedarLane) and Ficoll-Hypaque™ cell preparation tubes are recommended. The required reagent and separation protocol will depend on the species. Follow manufacturer's instructions.

#3 Red blood cells can be removed by lysis either before or after staining using BD PharM Lyse™ (Cat. No. 35221E), an ammonium chloride-based lysing solution.

#4 The optimal Ab concentration for cell staining is generally between 0.1 and 1.0 µg/10^6 cells, but must be determined for each cell type and antibody.

#5 To store samples for analysis, paraformaldehyde fixation may be used. However, it is possible that the quality of staining may be diminished by fixation. We do not recommend fixation of stained cells, except when the possibility of exposure to biohazardous material exists.

#6 When performing multi-color labeling, directly-conjugated mAbs can be added simultaneously, rather than sequentially.

#7 For reducing FcγR-mediated antibody binding to mouse or rat cells, the use of anti-mouse CD32/CD16 (Mouse Fc Block™, Cat No. 01241D) or anti-rat CD32 (Rat Fc Block™, Cat No. 22880D) is recommended. Fc Block™ can be added to cells (approximately 0.25 µg per million cells, 3 - 5 min, 4°C) before staining and need not be washed out prior to addition of primary mAb. However, it is important to verify that no secondary reagent used in the experiment will bind to the Fc block™.

#8 For very low-density cell surface markers, a three-step protocol may be used to amplify the staining. For three step staining, use purified primary antibody followed by biotinylated anti-Ig for the 2nd step and fluorochrome-conjugated avidin or streptavidin as the 3rd step.

#9 We find that SAv-PE, SAv-APC, and SAv-Cy-Chrome™ have greater fluorescent intensity than Avidin- or SAv-FITC conjugates and may provide better discrimination of cells that express an antigen at low-density.

B. Immunohistochemical Staining

Immunohistochemical techniques allow characterization of cells and their activities *in situ*. Preparation of frozen tissue sections is a gentler method than paraffin embedding and typically gives good preservation of cell morphology and antigen expression. The staining technique described here applies to 5 - 7 µm cryostat sections from frozen tissue. Please read the entire procedure before staining sections. Perform all incubations in a humid chamber at room temperature (RT) and do not allow sections to dry out during staining.

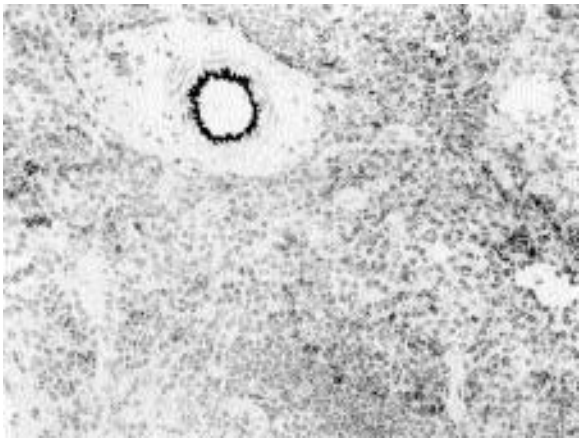


Figure 3. Expression of PECAM-1 (CD31) on rat endothelial cells. The frozen section of rat spleen was stained with anti-rat CD31 (clone TLD-3A12).

Protocol

1. After 5 - 7 µm cryostat sections are cut and applied to slides, the slides should be allowed to air dry at RT. Tissue sections can then be fixed by immersion in cold acetone for 10 min.

2. Label all slides using Secureline2 pen (most other pens are soluble in alcohol and/or xylene and will wash off during dehydration and clearing).

3. Rinse slides 2 - 3 times in PBS to remove frozen mounting media.

4. To quench endogenous peroxidase activity, apply 0.03% H_2O_2 solution in PBS for 10 min.

5. Rinse slides with PBS. Blot excess buffer from around the tissue section without touching the section itself.

6. Block with 5% normal serum for 20 min. (See note #1)

7. Apply enough properly diluted primary antibody to cover tissue sections on slide and incubate 1 hr at RT in a humid chamber.

8. Rinse slides in PBS three times, 2 min each wash. Blot excess buffer from around the specimen.

9. Apply properly diluted biotinylated secondary antibody and allow to incubate at RT for 30 min.

10. Wash slides as in step 8.

11. Apply streptavidin-horseradish peroxidase to each slide and incubate at RT for 30 min.

12. Wash slides as in step 8.

13. Prepare diaminobenzidine (DAB) according to manufacturer's specifications.

    SAFETY NOTE: *DAB is a suspected carcinogen and must be handled with care. Always wear gloves.*

14. Drain slides and place them on a flat surface.

15. Add DAB solution to each slide, making sure the entire section is covered by solution. Allow to incubate until color develops, up to 5 min.

16. Pick up each slide in the order that DAB was applied, drain excess DAB on paper towel and place in staining rack in a dish of water.

17. Rinse the slides well in water 3 times.

18. To counterstain slides:

    a) Cover section with Gill Hematoxylin and incubate for approximately 5 min. The duration of incubation will determine the intensity of counterstaining.

    b) Rinse gently in water.

    c) Dip twice in Bluing Reagent and rinse thoroughly in water.

19. Dehydrate stained slides by dipping in 2 changes of 95% ethanol followed by 3 changes of 100% ethanol, 2 min each.

20. Dip slides in 3 - 4 changes of xylene or xylene substitute, 2 min per wash.

21. Mount section under a glass coverslip in a mounting medium such as Permount.

Controls

Negative controls should include sections stained with the 2nd step reagent alone and/or cells exposed to an Ig isotype matched control for the primary Ab, followed by the 2nd step reagent.

Notes

#1 Blocking non-specific binding by pre-incubating the tissue section with serum is optional. To block, always use serum from the species in which the secondary Ab is made. Incubate for 10 - 30 min before application of the primary Ab. Remove blocking solution by tapping and blotting around section. Do not rinse prior to addition of primary Ab.

#2 If the chromagen used is not stable in alcohol, use aqueous mounting media and cover with cover slip. **DO NOT** dehydrate and clear slides as in Steps 19 and 20.

For a complete list of Immunohistochemistry reagents, please see the BD Biosciences Catalog or contact a technical service representative 877.232.8995

C. Immunofluorescent Labeling of Cells in Culture

Immunofluorescent (IF) labeling of cells in culture allows co-localization of specific adhesion molecules with sites of adhesion. Usually cells are grown (adherent cells) or plated (non-adherent cells) on glass coverslips coated with adhesion molecules, such as the BD BioCoat™ Extracellular Matrix Products (Table I, pg.3). Then cells on coverslips are immersed for 30 minutes in 3.7% formaldehyde and 0.2% Triton-X100 solution at 37°C. Alternatively, cells on coverslips are immersed in ice-cold methanol:acetone (1:1) for 10 minutes at -20°C, then air dried at room temperature. The latter fixation is ideal for preservation of the actin cytoskeleton.

Blocking and Staining Protocol

1. Place the coverslips cells-side-up on a piece of parafilm inside a petri dish. Rinse the cells with PBS, then cover the cells with blocking buffer (1% BSA in PBS) for 30 minutes at 37°C to minimize non-specific adsorption of the antibodies to the coverslip (50 – 100 µl is usually sufficient to completely cover the surface area).

2. Remove the blocking buffer by aspiration or by holding each coverslip on its edge with forceps and draining it onto a sheet of fiber-free paper.

3. Dilute primary antibody to 1.0 – 10 µg/ml in blocking buffer (optimal concentration will depend on several variables, such as the affinity of the antibody and the abundance of the antigen).

4. Distribute 50 – 100 µl of the primary antibody solution on each coverslip and incubate for 1 hour at room temperature.

5. Remove primary antibody, and wash coverslips three times in PBS, 5 minutes each wash.

6. Dilute the secondary antibodies to the appropriate concentration in blocking buffer (See manufacturer's recommendation). Add enough secondary antibody solution to cover the surface of each coverslip (usually 50 – 100 µl) and incubate for 1 hour at room temperature.

7. Remove the secondary antibody, and wash coverslips three times in PBS, 5 minutes each wash.

8. Invert each coverslip onto a slide containing 10 µl of mounting medum (VECTASHIELD mounting medum, Vector Laboratories).

9. Remove the excess mounting medum with fiber-free paper, without disturbing the coverslip. Seal the edges of each coverslip with regular transparent nail polish and allow to dry. This will provide semi-permanent preparations. The cells are now ready for microscopic viewing.

For additional information on immunofluorescent labeling, please see the BD Biosciences Catalog or contact a technical service representative at 877.232.8995

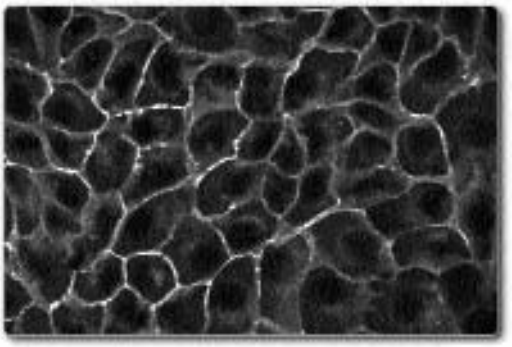


Figure 4. Immunofluorescent labeling of focal adhesion contacts in human fibroblasts using anti-paxillin clone 349 (Cat. No. P13520)

D. Immunoblotting

Immunoblotting is an effective method for analysis of proteins from a wide variety of sources, such as cells grown in culture or tissues. It allows detection of small quantities of antigen from complex lysates and a determination of relative molecular weight.

Sample Preparation

For Protein Concentration Determination of Cell Culture:

1. Decant medium from 10 cm dish of adherent cells, rinse plate rapidly with phosphate-buffered saline (PBS), and aspirate excess PBS.

2. Add 1 ml boiling lysis buffer (1% SDS, 1.0 mM sodium ortho-vanadate, 10 mM Tris pH 7.4).

3. Scrape cells from dish, transfer to a microcentrifuge tube, and boil for an additional 5 minutes. To reduce viscosity, the sample may be sonicated briefly or passed several times through a 26 gauge needle.

4. Centrifuge (16,000 × *g*, 15°C) the sample for 5 minutes to pellet insoluble material, then discard pellet. Dilute an aliquot of the cell lysate sample at least 10-fold for the BCA (Pierce) protein concentration assay (SDS concentration must be below 0.1% to avoid interference with the colorimetric reading).

For Protein Concentration Determination of Whole Tissue:

1. Rapidly homogenize every 0.25 g tissue in 3.5 ml of boiling lysis buffer (1% SDS, 1.0 mM sodium ortho-vanadate, 10 mM Tris pH 7.4).

2. Microwave for 10 – 15 seconds.

3. Centrifuge the homogenate (16,000 × *g*, 15°C) for 5 minutes to pellet insoluble material, then discard pellet.

4. Dilute an aliquot of the tissue lysate sample at least 10-fold for the BCA (Pierce) protein concentration assay.

Polyacrylamide Gel Electrophoresis

Guidelines for choosing the percent gel to be used for certain molecular weight proteins (based on 37:1 acralyamide: bis acraylamide ratio).

4 - 5% gels: > 250 kDa

7.5% gels: 250 - 120 kDa

10% gels: 120 - 40 kDa

13% gels: 40 - 15 kDa

15% gels: < 20 kDa

1. If not already in electrophoresis sample buffer, add an equal volume of 2X sample buffer (125 mM Tris pH 6.8, 4% SDS, 10% glycerol, 0.006% bromophenol blue, 1.8% β-mercaptoethanol) to all samples and boil for 3 – 5 minutes.

2. Apply 5 - 15 µg total protein of cell or tissue lysate to each well of a 0.75 – 1.0 mm thick gel. For thicker gels (1.5 mm thick), apply up to 25 - 40 µg in each well.

3. Electrophorese until the bromophenol blue in the samples reaches the bottom of the gel. Turn off power supply. Keep gels in running buffer until ready to transfer.

Protein Blotting

Wet Transfer

Note: Since extra negative charges are needed to reach 1 Amp in a wet transfer system, adjust the pH of the transfer buffer to approximately pH 8.0 using NaOH.

1. For transfer of proteins smaller than 20 kDa, transfer proteins from gel to PVDF (polyvinylidene difluoride) membrane at 1 Amp constant current for 45 minutes or equivalent (250 mAmp for 3 hours or 500 mAmp for 90 minutes) in transfer buffer (25 mM Tris, 190 mM glycine, 20% MeOH).

2. For transfer of proteins smaller than 120 kDa, transfer proteins from gel to PVDF membrane at 1 Amp constant current for 1 hour or equivalent (250 mAmp for 4 hours or 500 mAmp for 2 hours) in transfer buffer (25 mM Tris, 190 mM glycine, 20% MeOH).

3. For proteins larger than 120 kDa, transfer to PVDF membrane at 1 Amp constant current for 90 minutes or equivalent (250 mAmp for 6 hours or 500 mAmp for 3 hours) in transfer buffer + SDS (25 mM Tris, 190 mM glycine, 20% MeOH, 0.05% SDS).

4. For Proteins larger than 250 kDa, transfer to PVDF membrane at 1 Amp constant current for 1 hour and 45 minutes or equivalent (500 mAmp for 3.5 hours) in transfer buffer + SDS (25 mM Tris, 190 mM glycine, 20% MeOH, 0.05% SDS).

Semi-Dry Transfer

For transfer of proteins from 10% or 13% gels to PVDF membranes semi-dry transfer can also be used. Transfer proteins to PVDF membrane at 1.2 mAmp/cm^2 for 1 hour 45 minutes in transfer buffer (25 mM Tris, 190 mM glycine, 20% MeOH).

Blocking

For All Antibodies Except Phosphotyrosine Antibodies:

1. Remove the blot from the transfer apparatus or staining tray and immediately place into blocking buffer (5% non-fat dry milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20).

2. Incubate the blot for 30 minutes at 37°C, 1 hour at room temperature, or overnight at 4°C.

For Phosphotyrosine Antibodies:

1. Remove the blot from the transfer apparatus or staining tray and immediately place into blocking buffer (1% BSA, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20).

2. Incubate the blot for 30 minutes at 37°C, 1 hour at room temperature, or overnight at 4°C.

Antibody Incubation

1. Dilute the antibody in the corresponding blocking buffer at the recommended dilution.

2. Decant the blocking buffer from the blot, add the antibody solution, and incubate with agitation for 30 minutes at 37°C, one hour at room temperature, or overnight at 4°C.

3. Decant the primary antibody solution, add wash buffer (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20), and wash for 30 minutes with agitation, changing the wash buffer every 3 – 5 minutes.

4. Dilute the enzyme conjugated second antibody 1:2000 in wash buffer containing 5% non-fat dry milk (or 1% BSA for phosphotyrosine antibodies).

5. Decant the wash buffer, add the diluted enzyme conjugate and incubate with agitation for 30 minutes at 37°C or one hour at room temperature.

Substrate Incubation

1. Decant the secondary antibody solution, add wash buffer (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20), and wash for 30 minutes with agitation, changing the wash buffer every 3 – 5 minutes.

2. Decant wash buffer and place the blot in a plastic bag or clean tray containing chemiluminescent working solution (0.125 ml/cm^2). Rotate the bag or tray to allow the solution to cover the surface of the membrane for 1 – 5 minutes.

3. Remove blot from the bag or tray and place it between two pieces of write-on acetate transparency film. Smooth over covered blot to remove air bubbles and excess substrate.

4. Expose the blot to X-ray film or any sensitive screen. An initial exposure of 10 – 60 seconds is recommended for film.

For additional information on Western Blotting, please see the BD Biosciences Catalog or contact a technical service representative at 877.232.8995

Figure 5. Western Blot of E-cadherin from A431 Cells using clone 36 (Cat. No. C20820).

E. BD RiboQuant™ Assay and Product List

The ribonuclease protection assay (RPA) is a highly sensitive and specific method for the detection and quantitation of mRNA species. The assay was made possible by the discovery and characterization of DNA-dependent RNA polymerases from the bacteriophages SP6, T7 and T3, and the elucidation of their cognate promoter sequences. These polymerases are ideal for the synthesis of high-specific-activity RNA probes from DNA templates because they exhibit a high degree of fidelity for their promoters, polymerize RNA at a very high rate, efficiently transcribe long segments, and do not require high concentrations of rNTPs. Thus, a cDNA fragment of interest can be subcloned into a plasmid that contains bacteriophage promoters, and the construct can then be used as a template for synthesis of radiolabeled anti-sense RNA probes.

BD Biosciences Pharmingen's strategy for the development of multi-probe RPA systems is to generate a series of such templates, each of distinct length and each representing a sequence in a distinct mRNA species. The templates are then assembled into biologically relevant sets to be used by investigators for the T7 polymerase-directed synthesis of a high-specific-activity, [^{32}P]-labeled anti-sense RNA probe set. The probe set is hybridized in excess to target RNA in solution, after which free probe and other single-stranded RNA are digested with RNases.

The remaining "RNase-protected" probes are purified, resolved on denaturing polyacrylamide gels, and quantified by autoradiography or phosphorimaging. The quantity of each mRNA species in the original RNA sample can then be determined based on the intensity of the appropriately-sized, protected probe fragment.

Two distinct advantages of the multi-probe RPA approach are its sensitivity and its capacity to simultaneously quantify several mRNA species in a single sample of total RNA. This allows comparative analysis of different mRNA species within samples and, by incorporating probes for housekeeping gene transcripts, the levels of individual mRNA species can be compared between samples. Moreover, the assay is highly specific and quantitative due to the RNase sensitivity of mismatched base pairs and the use of solution-phase hybridization driven toward completion by excess probe. Lastly, the multi-probe RPA can be performed on total RNA preparations derived by standard methods from either frozen tissues or cultured cells, without further purification of poly-A + RNA.

BD RiboQuant™ Multi-Probe RNase Protection Assay is a complete system available for detecting and quantifying transcripts. The system includes:

- Multi-Probe Template Sets
- *In Vitro* Transcription Kit
- RPA Kit

Individual components may be purchased either separately or together as the RPA Starter Package (Cat. No. 45024K). The Starter Package includes one Template Set, the *In Vitro* Transcription Kit, and the RPA Kit.

1. Adhesion Template Sets

Human Angio-1 Multi-Probe Template Set **Cat. No. 556232**

Template	Probe	Protected
Flt-1	390	361
Flt-4	348	319
TIE	312	283
Thrombin Receptor	285	256
TIE2	255	231
CD31	231	202
Endoglin	210	181
Angiopoietin 1	192	163
VEGF	177	148
VEGF-C	165	136
L32	141	113
GAPDH	124	96

Human Angio-2 Multi-Probe Template Set — Cat. No. 556851

Template	Probe	Protected
Endothelin RA	429	400
Endothelin RB	393	364
Endothelin RB-like	354	325
Thrombin receptor	315	286
PAR2	282	253
PAR3	255	226
PAFR	231	202
H963 PAFR	210	181
PAFR-like	189	160
L32	141	112
GAPDH	126	97

Human Angio-3 Multi-Probe Template Set — Cat. No. 559900

Template	Probe	Protected
Edg1	312	283
Edg2	282	253
Edg3	255	226
GPR41	230	201
KIAA0001	209	180
FEG1	189	160
L32	141	113
GAPDH	124	97

Human ITG-1 Multi-Probe Template Set — Cat. No. 559928

Template	Probe	Protected
ITGA1 (α_1/CD49a)	459	430
ITGA2 (α_2/CD49b)	417	388
ITGA3 (α_3/CD49c)	378	349
ITGA4 (α_4/CD49d)	342	313
ITGA5 (α_5/CD49e)	309	280
ITGA6 (α_6/CD49f)	279	250
ITGA7 (α_7)	252	223
ITGA8 (α_8)	228	199
ITGA9 (α_9)	207	178
ITGAv (α_v/CD51)	189	160
ITGB1 (β_1/CD29)	174	145
L32	141	112
GAPDH	126	97

Human ITG-2 Multi-Probe Template Set — Cat. No. 550355

Template	Probe	Protected
ITGAe (α_e/CD103)	417	388
ITGB4 (β_4/CD104)	378	349
ITGA4 (α_4/CD49d)	342	313
ITGB5 (β_5)	309	280
ITGA6 (α_6/CD49f)	279	250
ITGB6 (β_6)	252	223
ITGB7 (β_7)	228	199

Human ITG-2 Multi-Probe Template Set (*continued*) **Cat. No. 550355**

Template	Probe	Protected
ITGB8 (β_8)	207	178
ITGAv (α_v/CD51)	189	160
L32	141	112
GAPDH	126	97

Human ITG-3 Multi-Probe Template Set **Cat. No. 550356**

Template	Probe	Protected
ITGA2b (α_{11b}/CD41)	459	430
ITGAd (α_d/CD11d)	417	388
ITGAL (α_L/CD11a)	378	349
ITGAm (α_m/CD11b)	342	313
ITGAx (α_x/CD11c)	309	280
ITGB2 (β_2/CD18)	279	250
ITGB3 (β_3/CD61)	252	223
ITGB7 (β_7)	228	199
ITGB8 (β_8)	207	178
ITGAv (α_v/CD51)	189	160
L32	141	112
GAPDH	125	97

Human MMP-1 Multi-Probe Template Set **Cat. No. 551274**

Template	Probe	Protected
MMP8	459	430
MMP12	378	349
TIMP3	342	313
MMP1	309	280
MMP2	279	250
MMP7	252	223
MMP9	228	199
MMP13	174	145
TIMP2	155	126
L32	141	160
GAPDH	125	97

Human MMP-2 Multi-Probe Template Set5 **Cat. No. 551275**

Template	Probe	Protected
MMP8	459	430
MMP12	378	349
TIMP3	342	313
MMP1	309	280
MMP2	279	250
MMP3	252	223
MMP14	207	178
TIMP1	169	140
TIMP2	155	126
L32	141	160
GAPDH	125	97

Mouse Angio-1 Multi-Probe Template Set — Cat. No. 551418

Template	Probe	Protected
Flt-1	390	361
Flt-4	348	319
TIE	312	283
TIE2	285	256
Thrombin Receptor	260	231
CD31	231	202
VEGF-C	210	181
Endoglin	192	163
VEGF	177	148
Angiopoietin-1	165	136
L32	141	112
GAPDH	125	97

Mouse MMP-1 Multi-Probe Template Set — Cat. No. 551276

Template	Probe	Protected
MMP1	459	430
MMP2	417	388
MMP3	378	349
MMP9	342	313
MMP8	309	280
TIMP4	251	222
TIMP3	228	199
TIMP1	207	178
TIMP2	187	158
L32	141	112
GAPDH	125	97

Mouse MMP-2 Multi-Probe Template Set — Cat. No. 551277

Template	Probe	Protected
MMP1	459	430
MMP12	417	388
MMP3	378	349
MMP9	342	313
MMP8	309	280
MMP7	279	250
TIMP3	228	199
TIMP1	207	178
TIMP2	187	158
GAPDH	125	97

Custom Adhesion template sets are available through the BD RiboQuant™ custom template service program. Please contact a technical service representative at 877.812.8995 about custom BD RiboQuant™ orders

Template	Probe	Protected
human CD11b	284	255
human CD31	231	202
human Collagen Type I α2	256	228
human Collagen Type III α1	231	204
human Collagen Type IV α1	210	181
human E-Cadherin	389	360
human Endoglin	210	181
human Hyaluronan Synthase I	285	257
human Hyaluronan Synthase II	256	230
human Hyaluronan Synthase III	236	208
human ICAM-1	284	255
human Osteocalcin	156	127
human Plasminogen	191	162
human E-Selectin	257	230
human P-Selectin	256	227
human Syndecan-1	349	320
human VCAM-1	316	288
human vWF	315	286
mouse CD11b	259	230
mouse CD31	232	202
mouse Collagen	231	205
mouse Collagen α1 Type II	256	228
mouse Collagen α1 Type X	286	257
mouse Endoglin	192	163
mouse ICAM-1 (A)	258	229
mouse ICAM-1 (B)	435	406
mouse MADCAM	316	287
mouse Osteocalcin	210	181
mouse Osteopontin	351	322
mouse E-Selectin	351	321
mouse Tenascin	172	144
mouse Thrombospondin	435	406
mouse VCAM-1	285	256

2. *In Vitro* Transcription Kit

The *In Vitro* Transcription Kit (**Table III**) is optimized for the efficient synthesis of high-specific-activity riboprobes from the BD Pharmingen™ Multi-Probe Template Sets. Each kit contains sufficient reagents for 25 transcription reactions (Cat. No. 45004K).

Table III. *In Vitro* Transcription Kit Components

Reagent	Conc.	Volume	Reactions	Cat. No.
GTP/ATP/CTP/ UTP (GACU Pool)	GAC:2.75 mM each, U:61 mM	25 µl	25	45005Z
DTT	100 mM	50 µl	25	45006Z
Transcription Buffer	5X	100 µl	25	45007A
RNasin	40 U/ml	25 µl	25	45008Z
T7 RNA Polymerase	20 U/ml	25 µl	25	45009Z
RNase-free DNase	1 U/ml	50 µl	25	45010Z
Yeast tRNA	2 mg/ml	50 µl	25	45011Z
EDTA	20 mM	650 ml	25	45012Z
Ammonium Acetate	4M	1.3 ml	25	45013Z

RPA Kit

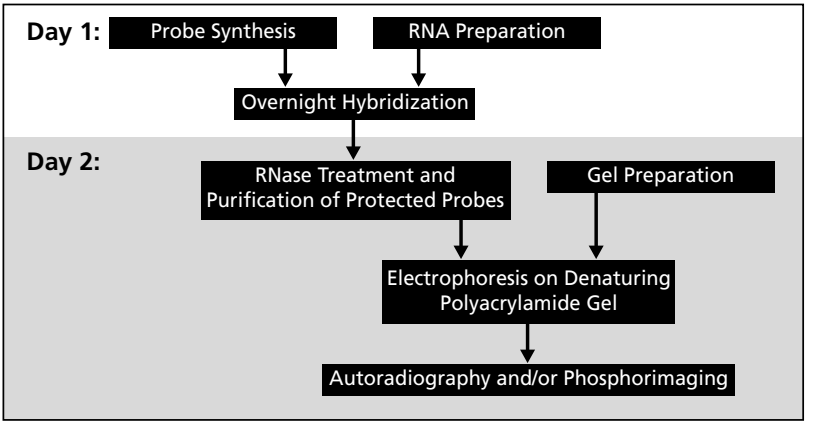
The RPA Kit contains reagents necessary to perform the RPA procedure as outlined in the Standard RPA Protocol. Each kit contains sufficient reagents for the analysis of 200 RNA samples (Cat. No. 45014K).

Table IV. RPA Kit Components

Reagent	Conc.	Volume	Reactions	Cat. No.
Hybridization Buffer	1X	3.6 ml	200	45015A
RNase Buffer	1X	25 ml	200	45016A
RNase A + T1 Mix	A:80 ng/ml T1:250 U/ml	60 µl	200	45017Z
Proteinase K Buffer	1X	3.9 ml	200	45018A
Proteinase K	10 mg/ml	300 µl	200	45019Z
Yeast tRNA	2 mg/ml	300 µl	200	45020Z
Ammomium Acetate	4M	24 ml	200	45021Z
Loading Buffer	1X	1.3 ml	200	45022Z

RPA Protocol Overview

In all steps of the protocol, standard precautions should be used to avoid RNase contamination and exposure of personnel to radioactivity. Typically, the probe synthesis is performed during the afternoon of Day 1, hybridizations are incubated overnight, and RNase treatments and gel electrophoresis are performed early on Day 2.



Please visit www.bdbiosciences.com or refer to the BD RiboQuant™ Instruction manual 6th edition for a detailed protocol.

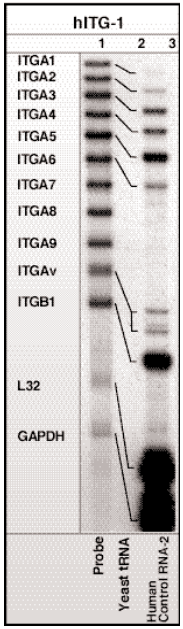


Figure 6. Samples of total RNA from various sources were analyzed for distinct mRNA species by using BD RiboQuant™ Multi-Probe Ribonuclease Protection Assay System with the hITG-1 Multi-Probe Template Set. The autoradiogram from this analysis shows the hITG-1 Multi-Probe Template Set not treated with RNases (Lane 1). Also shown are the corresponding RNase-protected probes following hybridization with Yeast tRNA (2 µg, Lane 2) and RNA from Human Control NA-2 (10 µg, Lane 3). Note that each probe band (Lane 1) migrates more slowly than its protected band (Lane 3); this is due to flanking sequences in the probe that are not protected by mRNA.

IV. Quantitation of Cell Adhesion to Immobilized Ligand

A variety of techniques have been developed to allow characterization of the molecular mechanisms involved in cell adhesion. Many purified ligands can be directly coated onto the surface of microwell plates, and methods using multiwell plates offer the advantage of determination of cell adhesion under a number of different experimental conditions. In most microwell plate-based adhesion assays, cells are allowed to bind to immobilized ligands and then non adherent cells are removed by gentle washing. The remaining adherent cells can be quantitated using many different methods. The simplest method of determining the number of adherent cells is to count them by microscopic examination. For the vast majority of applications, a less time consuming and labor intensive method is recommended. Here, protocols for colorimetric, fluorescence-based and radioisotope-based assays are described. The selection of protocols described here is not intended to be all inclusive, but rather, to provide a sampling of some of the more commonly used techniques. Also, the relative advantages and disadvantages of these different assays are discussed briefly.

A. Colorimetric Cell Adhesion Assays

There are a number of colorimetric methods which can be used for quantitating adherent cells in microwell plates. Two methods are presented here. One is based on staining cells with the dye crystal violet and the second is based on quantitation of the cellular enzyme acid phosphatase.

Protocols

1. Coat plate.

   a) Coat a 96 well flat bottom microwell plate with protein diluted in PBS, pH 7.4. (See note #1 pg.30) Add 0.05 ml volume per well and tap plate to disperse.

   b) Cover plate and incubate at room temperature (RT) for 1.5 hours or at 4°C overnight.

   c) To block any remaining protein binding sites on the plate, remove coating solution and add 0.15 ml of 1.0% BSA in PBS per well. In each assay, include wells coated with 1.0% BSA alone so that the background level of cell adhesion can be determined. Incubate at RT for 30 min.

   d) Immediately prior to adding cells, wash wells three times with 0.15 ml RT PBS. When adding PBS, use a multichannel pipetor to gently dispense PBS against sides of well rather than directly onto the bottom surface. To remove PBS, invert plate and "flick" out contents. Do not let the wells dry out during the assay.

2. Allow cells to adhere to immobilized ligand.

   a) Harvest cells and prepare a single cell suspension in RPMI with 2% fetal bovine serum (RPMI + 2% FBS). (See note #2 pg.30) If cells are prepared from homogenized tissue, pass the suspension through a 70 µm nylon mesh to remove large cell aggregates. Cells may be stimulated to induce adhesion.

   b) Add 0.1 ml cell suspension to each well and incubate at 37°C for 1 - 2 hours. Optimum incubation time will vary with cell type, activation state and the immobilized ligand.

3. After cells have been allowed to adhere, gently wash plate 5 times with RT PBS to remove non adherent cells. To avoid displacing cells, do not forcefully pipet directly onto the well surface. (See note #3 pg.30)

4. Detection of adherent cells by staining with crystal violet: Adherent cells can be detected by staining with the dye crystal violet.[1,2] In this method, after the non-adherent cells are washed away in step 3 above, the remaining cells are

fixed with ethanol and stained with crystal violet. Excess dye is removed by washing with dH_20 and the dye absorbed by adherent cell nuclei is extracted with Triton X.

a) Add 0.05 ml of 96% ethanol per well and incubate at RT for 10 min.

b) Add 0.05 ml 0.1% crystal violet per well and incubate at RT for 30 min.

c) Wash wells extensively with dH_20 to remove excess stain.

d) Add 0.05 ml 0.2% Triton X in dH_20 to lyse cells and read absorbance at 570 nm.

A commonly used modification of this assay uses fixation with glutaraldehyde instead of ethanol and extraction of crystal violet with acetic acid instead of Triton X-100.[3]

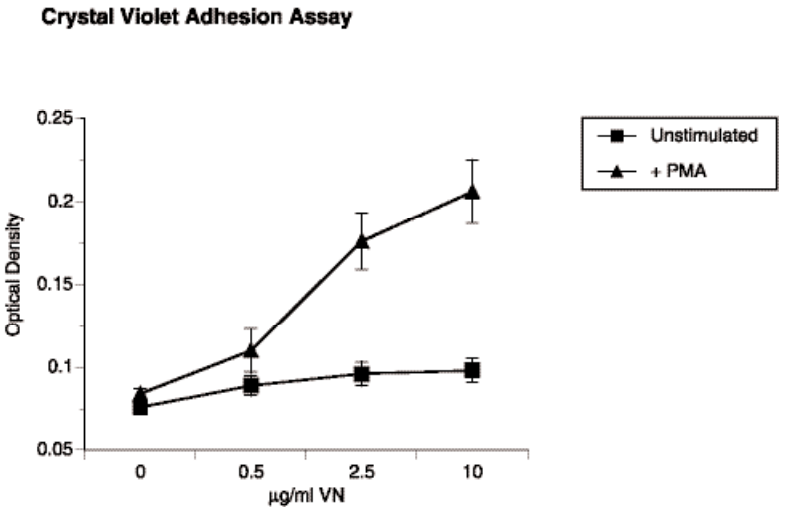


Figure 7. Crystal Violet Adhesion Assay. Mouse splenocytes were activated with PMA and cultured in wells coated with BSA or the indicated concentrations of human vitronectin (VN). After nonadherent cells were washed away, adherent cells were stained with crystal violet.

5. Detection of adherent cells by measurement of acid phosphatase activity. Adherent cells can be quantitated by measuring the activity of acid phosphatase, an enzyme which is stable, independent of activation and is not released.[4] After non-adherent cells are washed out of wells in step 3 above, the remaining cells are solubilized with Triton X-100, and acid phosphatase activity is measured by addition of the substrate p-nitro-phenyl phosphate.

   a) Make up fresh Lysis Buffer for each assay: To 50 mM sodium acetate stock solution pH 5.0, add 12 mM p-nitro-phenyl phosphate (Sigma) and 0.2% Triton X-100 in dH_20.

   b) Add 0.1 ml of Lysis Buffer per well and incubate 1 hour at 37°C, covered.

   c) Add 0.05 ml 1N NaOH per well.

   d) Determine OD with spectophotometric plate reader at 405 nm. Include a well with cell free lysis buffer plus NaOH as a blank.

   e) To determine percent cell adhesion, calculate cell number from OD using the equation generated with a standard curve.

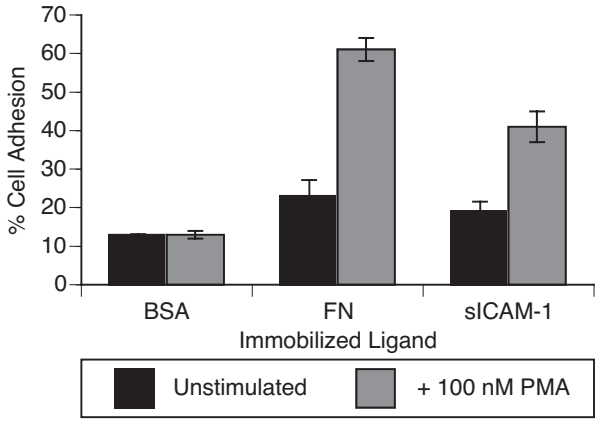


Figure 8. Acid phosphatase adhesion assay. TK1 cells were stimulated with PMA and incubated in wells coated with BSA, fibronectin (FN), or soluble ICAM-1 (sICAM-1). Cell adhesion was determined by quantitation of acid phosphatase in adherent cells.

B. Radiometric Determination of Cell Adhesion

Adherent cells can be detected based on the presence of an incorporated radioisotope such as ^{3}[H]thymidine or ^{51}Cr. In the protocol detailed below, the cells are labeled with ^{3}[H]thymidine prior to initiation of adhesion to the coated microtiter wells. Use appropriate safety measures when handling and disposing of all radioactive materials.

Protocol

1. Coat plate. Coat the microwell plate as described in protocol for Colorimetric Cell adhesion Assays, Step 1 pg.24.

2. Prepare labeled cells.

   a) Cells should be labeled by a 12 - 16 hour incubation in complete medium at 37°C with 1 µCi ^{3}[H]thymidine per 2 - 5 × 10^{6} cells (See note # 4 pg.30).

   b) Wash cells three times in RPMI by centrifuging at 350 × *g* and resuspend at 2 × 10^{6} in RPMI + 2% FCS (See note #2 pg.30).

3. Allow cells to adhere to immobilized ligand. Add 0.1 ml cells per well and incubate at 37°C for 1 - 2 hours. Optimum incubation time will vary with cell type, activation state and the immobilized ligand.

   a) Wash plate. When cells have been allowed to adhere, gently wash plate 4 times with RT PBS to remove non adherent cells. To avoid displacing cells, do not forcefully pipetting directly onto the well surface.

4. Determine counts per minute (cpm) of adherent cells.

   a) Add 0.1 ml of 3% SDS to each well and incubate at RT for 15 min.

   b) Transfer contents to scintillation vials and determine cpm.

   c) In each assay, cpm in 0.1 ml of cells (total cpm) and cpm released into the supernatant during the assay (spontaneous cpm) must be determined. Percent cells bound is calculated as 100 × cpm bound/total cpm - spontaneous cpm.

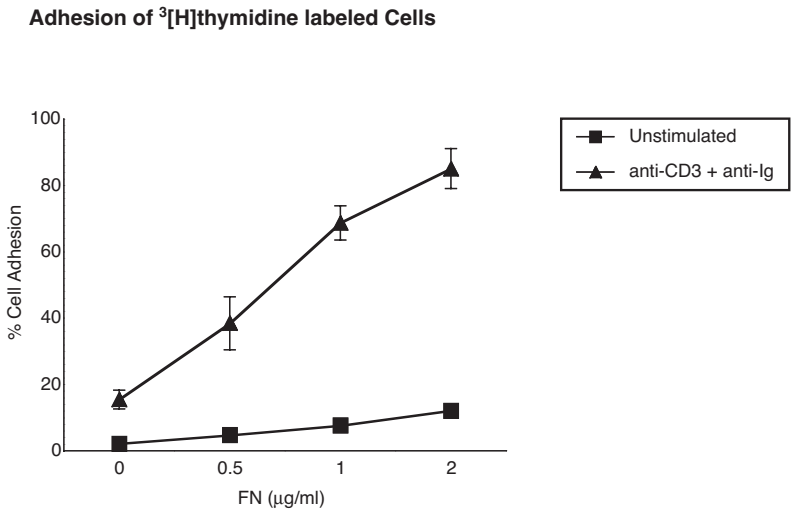


Figure 9. Radiometric Adhesion Assay. Mouse cytotoxic T cell clone 11 was labeled with ^{3}[H]thymidine. Labeled cells were either unstimulated or stimulated with anti-CD3/anti-Ig and then incubated in wells coated with the indicated concentration of fibronectin (FN). Cell adhesion was determined by quantitation of counts per minute in adherent cells.

C. Fluorescence-based Cell Adhesion Assay

Fluorescence-based adhesion assays involve labeling cells with a fluorescent dye, allowing cells to adhere to immobilized ligand, washing away non-adherent cells, and measuring the fluorescent signal of adherent cells with a fluorimetric plate reader (Figure 10). Cells are labeled with fluorescein derivatives such as bis-carboxymethyl-carboxyfluorescein (BCECF) and calcein in their acetoxymethylester (AM) forms. The fluorescent dyes are taken up by cells and hydrolyzed by cellular esterases. Calcein is recommended due to its pH-independence, superior retention by cells and photostability.[5] Also, calcein does not affect lymphocyte adhesion, proliferation, or granulocyte chemotaxis.[6]

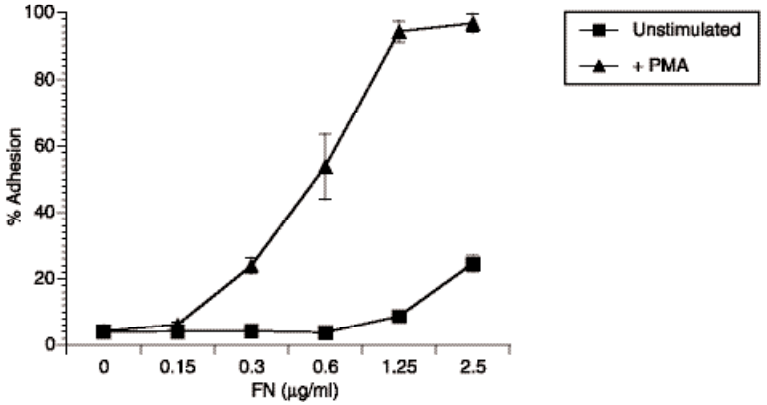


Figure 10. Fluorimetric Adhesion Assay. Jurkat cells were labeled with calcein-AM. Labeled cells were either unstimulated or stimulated with PMA and incubated in wells coated with the indicated concentrations of fibronectin (FN). Cell adhesion was determined by measuring fluorescent units in adherent cells.

Protocol

1. Coat plate: Prepare plate as described in protocol for Colorimetric Cell adhesion Assays, Step 1 pg.24.

2. Prepare labeled cells.

   a) Harvest cells and resuspend cells in RPMI (no additives) at 1×10^7 per ml.

   b) Add calcein-AM to give 2 mM concentration (See notes #5 and #6 pg.30).

   c) Incubate at RT in the dark for 30 minutes. Gently invert tube to mix at 15 min.

   d) Wash cells three times to remove excess calcein. To wash, centrifuge at $350 \times g$ and resuspend in cold RPMI.

   e) Resuspend the cell at 2×10^6 per ml in RPMI + 2% FCS.

3. Allow cells to adhere to immobilized ligand. Add 0.1 ml cells per well and incubate at 37°C for 1 - 2 hours. Optimum incubation time will vary with cell type, activation state and the immobilized ligand.

4. Gently wash plate 3 - 5 times with RT RPMI to remove non adherent cells. To avoid displacing cells, do not forcefully pipet directly onto the well surface (See note #3 below).

5. Determine fluorescent units (FU).

   a) Add 0.1 ml of RPMI + 2% FCS (See note #7 pg.31).

   b) Read plate in a fluorescent plate reader at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

   c) In each assay, fluorescence units (FU) in 0.1 ml of cells (total) and FU released into the supernatant during the assay (spontaneous) must be determined. Percent cells bound can be calculated as 100 × bound FU/total FU - spontaneous FU.

Notes

#1 The concentration of protein required to efficiently coat the well surface will vary, but is generally between 2 and 10 µg/ml. Note that immobilization of very small molecules such as peptides may result in loss of available binding sites. In some cases it may be necessary to couple the peptide to a carrier protein such as ovalbumin.

#2 The optimum cell number will depend on cell size, the propensity for spreading and the type of assay. Generally, it is best to select a cell number that results in a single layer of cells covering most of the well surface.

#3 If adherent cells are too easily detached during washing step, it may be preferable to gently immerse the plate in wash buffer rather than dispensing the wash buffer with a multi-channel pipetor.

#4 For ^{3}[H]thymidine labeling, the optimum culture medium, cell density, and length of incubation will depend on the cell type.

#5 Calcein-AM is diluted in DMSO and stored according to the manufacturer's instructions. We recommend that the concentration of DMSO added to cells not exceed 0.05%.

#6 The concentration of the fluorochrome calcein-AM required for optimum labeling should be determined for each cell type. It is very important to verify that the concentration of calcein selected results in good labeling and minimal leakage of the fluorochrome from the cells during the adhesion assay.

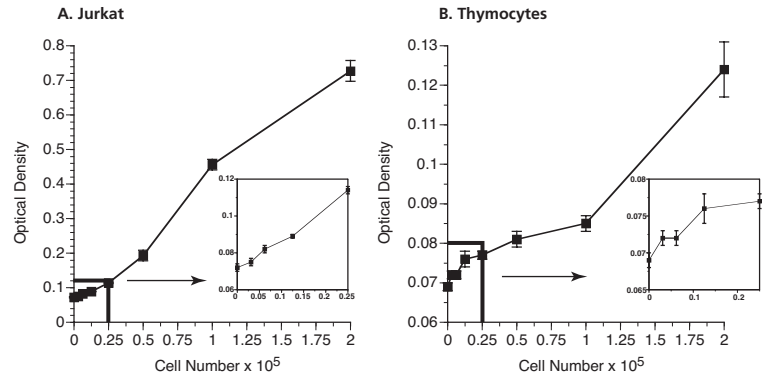
#7 It has been reported that the presence of phenol red interferes with detection of calcein fluorescence. We have found this effect to be minimal. However, to avoid any reduction in signal, HBSS + 2 - 10% FCS can be substituted for RPMI + 2% FCS. The same medium or buffer selected for the assay must be used in both the experimental and control (total fluorescent label and spontaneously released fluorescent label) wells.

D. Comparison of Assay Methods

For any study, we recommend that preliminary experiments be done to select the optimum assay method and conditions. Some assay characteristics to be considered are listed below.

Crystal Violet Staining

Staining adherent cells with crystal violet is simple and easily done. It should be noted that some bound cells may be lost due to the staining and washing steps. A disadvantage of this method is that it does not allow quantitation of cell number in reference to a standard curve. Changes in OD measure qualitative variation in adhesion rather than the number of bound cells.[4]



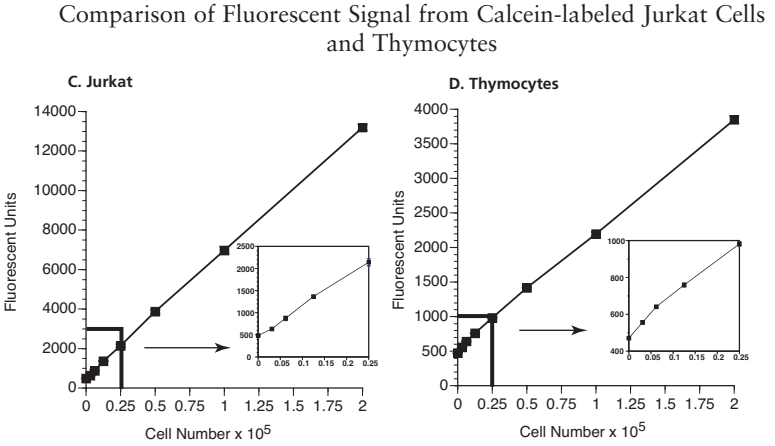


Figure 11. Comparison of signals generated by acid phosphatase assay and fluorochrome labeling of Jurkat and thymocytes. To quantitate acid phosphatase activity, Jurkat cells (A) and mouse thymocytes (B) were suspended at the indicated concentrations and the acid phosphatase present in the total cell number per well was determined. To determine the fluorescent signal generated by labeling, Jurkat (C) and mouse thymocytes (D) were labeled with calcein-AM. Cells were then washed and resuspended at the indicated concentrations. Fluorescent units per well were determined in a fluorimetric plate reader.

Acid Phosphatase Assay

Quantitation of acid phosphatase is a simple and easy method for determining cell adhesion. Because it is based on measurement of a cellular component, no labeling of cells is required. The acid phosphatase assay is relatively sensitive and has been reported to detect as few as 2×10^4 neutrophils per well[4] and 3×10^5 platelets per well.[7] However, the signal generated will vary between different types of cells and this variation should be considered when selecting an assay method. For example, thymocytes generate a relatively weak signal in this assay, as shown in **Figure 11, B.**

Fluorimetric Assay

Detection of fluorochrome-labeled adherent cells is rapid, inexpensive and highly sensitive. It has been reported to detect as few as 1000 labeled lymphocytes bound to an endothelial cell monolayer.[8] The fluorescent dye can be easily taken up by cells, including resting lymphocytes. The method can be used for analyzing adhesion to immobilized ligand and adhesion to cell monolayers. A disadvantage of this method is that cells must be labeled. Controls must be included to monitor determine leakage of the dye as significant leakage of cell-associated label is associated with some cell types.[9] Also, a fluorimetric plate reader is required.

Radiometric Assay

Measurement of radiolabeled cells is very sensitive, but it has the disadvantage of requiring the expense and safety hazards associated with radioisotopes. In addition, cells such as resting lymphocytes are not well labeled with radioisotopes such as ^{3}[H]thymidine. Quantitation of bound cells requires the use of a scintillation counter.

E. Quantitation of Cell-Cell Adhesion

Two of the protocols for measuring cell adhesion to immobilized ligand described above can be adapted for analysis of adhesion to a cell monolayer. Cells may be labeled with either a fluorochrome or with a radioisotope prior to incubation on an monolayer of unlabeled cells. Unbound cells are then washed away, and the number of labeled cells which have adhered to the monolayer is quantitated with either a fluorimetric plate reader or a scintillation counter as described above.

V. Antibody Product List

Human Adhesion Molecules - Selectins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD62E	Hu	10E9.6	Rat (Lewis) IgG_{2a}, κ	Purified	1.0 ml	550290
		68-5H11	Mouse IgG_1, κ	Purified	0.1 mg	555648
				Biotin	0.1 mg	555649
CD62L	Hu	Dreg 56	Mouse IgG_1, κ	NA/LE	0.5 mg	555541
				Purified	0.1 mg	555542
				Purified	1.0 ml	550288
				FITC	100 tests	555543
				PE	100 tests	555544
				Cy-Chrome	100 tests	555545
				APC	100 tests	559772
		SK11	Mouse IgG_{2a}	Purified	0.2 mg	559050
CD62P	Hu	AC1.2	Mouse IgG_1	NA/LE	0.5 mg	550971
				Purified	0.1 mg	556087
		AK-4	Mouse IgG_1, κ	Purified	0.1 mg	555522
				FITC	100 tests	555523
				PE	100 tests	555524

Human Adhesion Molecules - Ig Superfamily

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD2	Hu	RPA-2.10	Mouse (Lewis) IgG_1, κ	NA/LE	0.5 mg	555323
				Purified	0.1 mg	555324
				Biotin	100 tests	555325
				FITC	100 tests	555326
				PE	100 tests	555327
				Cy-Chrome	100 tests	555328
		S5.2	Mouse (Lewis) IgG_{2a}	Purified	0.2 mg	559034
CD22	Hu	HIB22	Mouse (Lewis) IgG_1, κ	Purified	0.1 mg	555423
				FITC	100 tests	555424
				Cy-Chrome	100 tests	555425
CD31	Hu	WM59	Mouse (Lewis) IgG_1, κ	Purified	0.1 mg	555444
				FITC	100 tests	555445
				PE	100 tests	555446
CD48	Hu	TU145	Mouse (Lewis) IgM, κ	Purified	0.1 mg	555758
				FITC	100 tests	555759
CD50	Hu	TU41	Mouse (Lewis) IgG_{2b}, κ	Purified	0.1 mg	555957
				FITC	100 tests	555958
CD54	Hu	28	Mouse IgG1	Purified	50 µg	611704
				Purified	150 µg	611705
		HA58	Mouse (Lewis) IgG_1, κ	NA/LE	0.5 mg	555509
				Purified	0.1 mg	555510
				PE	100 tests	555511
				Cy-Chrome	100 tests	555512
				APC	100 tests	559771
		LB-2	Mouse (Lewis) IgG_{2b}	Purified	0.2 mg	559047
CD56	Hu	B159	Mouse (Lewis) IgG1, κ	NA/LE	0.5 mg	555513
				Purified	0.1 mg	555514
				Biotin	100 tests	555515
				PE	100 tests	555516
				Cy-Chrome	100 tests	555517
				APC	100 tests	555518
		MY31	Mouse (Lewis) IgG_1	Purified	0.2 mg	559049
		NCAM16.2	Mouse (Lewis) IgG_{2b}	Purified	0.2 mg	559043
CD58	Hu	1C3	Mouse (Lewis) $IgG2_a$, κ	Purified	0.1 mg	555919
				FITC	100 tests	555920
				PE	100 tests	555921
CD106	Hu	51-10C9	Mouse (Lewis) IgG_1, κ	Purified	0.1 mg	555645
				Biotin	0.1 mg	556911
				PE	100 tests	555647
CD147	Hu	HIM6	Mouse (Lewis) IgG_1, κ	Purified	0.1 mg	555961
				FITC	100 tests	555962
Contactin	Hu, Ms, Rat	41	Mouse IgG_1	Purified	50 µg	610578
				Purified	150 µg	610579
L1	C, Hu, Ms, Rat	17	Mouse IgG_{2b}	Purified	50 µg	610302
				Purified	150 µg	610303
LAR	Hu, Ms, Rat	7	Mouse IgG_{2a}	Purified	50 µg	610350
				Purified	150 µg	610351
MCAM	Hu	1	Mouse IgG_1	Purified	50 µg	611208
				Purified	150 µg	611209
RNCAM	Hu, Ms, Rat	44	Mouse IgG_1	Purified	50 µg	611092
				Purified	150 µg	611093
RPTP α	Hu	21	Mouse IgM	Purified	50 µg	610348
				Purified	150 µg	610349

Human Adhesion Molecules - Ig Superfamily (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
RPTP β	Hu, Ms, Rat	12	Mouse IgG_1	Purified	50 µg	610179
				Purified	150 µg	610180

Human Adhesion Molecules - Integrins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD11a	Hu	27	Mouse IgG_1	Purified	50 µg	610826
				Purified	150 µg	610827
	Hu	G25.2	Mouse IgG_{2a} (Sprague-Dawley)	Purified	0.2 mg	559035
		G43-25B	Mouse IgG_{2a}, κ (Sprague-Dawley)	NA/LE	0.5 mg	555377
				Purified	0.1 mg	555378
				FITC	100 tests	555379
				PE	100 tests	555380
		HI111	Mouse IgG_1, κ (Sprague-Dawley)	NA/LE	0.5 mg	555381
				Purified	0.1 mg	555382
				FITC	100 tests	555383
				PE	100 tests	555384
				APC	100 tests	559875
CD11b (Mac-1)	Hu	ICRF44 (44)	Mouse IgG_1, κ (Sprague-Dawley)	NA/LE	0.5 mg	555385
				Purified	0.1 mg	555386
				Biotin	100 tests	555387
				PE	100 tests	555388
				Cy-Chrome	100 tests	555389
				APC	100 tests	550019
CD11c	Hu	B-ly6	Mouse IgG_1, κ (Sprague-Dawley)	NA/LE	0.5 mg	555390
				Purified	0.1 mg	555391
				PE	100 tests	555392
				Cy-Chrome	100 tests	551077
				APC	100 tests	559877
CD18	Hu	6.7	Mouse IgG_1, κ (Sprague-Dawley)	Purified	0.1 mg	555922
				FITC	100 tests	555923
				PE	100 tests	555924
				Cy-Chrome	100 tests	557528
		L130	Mouse IgG_1 (Sprague-Dawley)	Purified	0.1 mg	556084
CD29	Hu	18	Mouse IgG_1	Purified	50 µg	610467
				Purified	150 µg	610468
		HUTS-21	Mouse IgG_{2a}, κ (Sprague-Dawley)	NA/LE	0.5 mg	556047
				Purified	0.1 mg	556048
				PE	100 tests	556049
		mAb13	Rat IgG_{2a} (Sprague-Dawley)	Purified	0.2 mg	559056
		3-Mar	Mouse IgG_1, κ (Sprague-Dawley)	Purified	0.1 mg	555442
				PE	100 tests	555443
				Cy-Chrome	100 tests	559882
				APC	100 tests	559883
CD41a	Hu	HIP8	Mouse IgG_1, κ (Sprague-Dawley)	Purified	0.1 mg	555465
				FITC	100 tests	555466
				PE	100 tests	555467
				Cy-Chrome	100 tests	559768
				APC	100 tests	559777

Human Adhesion Molecules - Integrins (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD41b	Hu	HIP2	Mouse IgG_3, κ	Purified	0.1 mg	555468
			(Sprague-Dawley)	FITC	100 tests	555469
CD49a	Hu	SR84	Mouse (Lewis) IgG_1	Purified	0.1 mg	559594
				PE	100 tests	559596
CD49b	Hu	2	Mouse IgG_{2a}	Purified	50 µg	611016
				Purified	150 µg	611017
		12F1-H6	Mouse (Lewis) IgG_{2a}, κ	Purified	0.1 mg	555668
				PE	100 tests	555669
		AK-7	Mouse (Lewis) IgG_1, κ	NA/LE	0.5 mg	555496
				Purified	0.1 mg	555497
				FITC	100 tests	555498
CD49c	Hu	C3 II.1	Mouse (Lewis) IgG_1, κ	Purified	0.1 mg	556024
				PE	100 tests	556025
CD49d	Hu	9F10	Mouse (Lewis) IgG_1, κ	NA/LE	0.5 mg	555501
				Purified	0.1 mg	555502
				PE	100 tests	555503
				APC	100 tests	559881
CD49e	Hu	1	Mouse IgG_{2a}	Purified	50 µg	610633
				Purified	150 µg	610634
		IIA1	Mouse (Lewis) IgG_1, κ	NA/LE	0.5 mg	555614
				Purified	0.1 mg	555615
				Biotin	100 tests	555616
				PE	100 tests	555617
		VC5	Mouse (Lewis) IgG_1, κ	NA/LE	0.5 mg	555650
				Purified	0.1 mg	555651
				Biotin	100 tests	555652
CD49f	Hu, Ms	GoH3	Rat IgG_{2a}, κ	Purified	0.1 mg	555734
			(Sprague-Dawley)	FITC	100 tests	555735
				PE	100 tests	555736
CD51	Dog, Hu, Ms, Rat	21	Mouse IgG_1	Purified	50 µg	611012
				Purified	150 µg	611013
CD51/CD61	Hu	23C6	Mouse IgG_1, κ	Purified	0.1 mg	555504
			(Sprague-Dawley)	FITC	100 tests	555505
				PE	100 tests	550037
CD61	Hu	1	Mouse IgG_1	Purified	50 µg	611140
				Purified	150 µg	611141
		VI-PL2	Mouse IgG_1, κ	Purified	0.1 mg	555752
			(Sprague-Dawley)	FITC	100 tests	555753
				PE	100 tests	555754
CD104	Hu	7	Mouse IgG_1	Purified	50 µg	611232
				Purified	150 µg	611233
		439-9B	Rat IgG_{2b}, κ	Purified	0.1 mg	555719
			(Sprague-Dawley)	PE	100 tests	555720
		450-11A	Mouse IgG_1, κ (Sprague-Dawley)	Purified	0.1 mg	555722
		450-9D	Mouse IgG_1, l (Sprague-Dawley)	Purified	0.1 mg	555721
Integrin β7	Hu	FIB504	Rat IgG_{2a}, κ	Purified	0.1 mg	555943
			(Sprague-Dawley)	PE	100 tests	555945

Human Adhesion Molecules - Cadherins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
Cadherin-5	Hu	75	Mouse IgG_1	Purified	50 µg	610251
				Purified	150 µg	610252
E-Cadherin	Hu, Rat	34	Mouse IgG_{2b}	Purified	50 µg	610404
				Purified	150 µg	610405
E-Cadherin	Hu, Ms, Rat	36	Mouse IgG_{2a}	Purified	50 µg	610181
				Purified	150 µg	610182
N-Cadherin	C, Hu, Ms, Rat	32	Mouse IgG_1	Purified	50 µg	610920
				Purified	150 µg	610921
P-Cadherin	Hu, Rat	56	Mouse IgG_1	Purified	50 µg	610227
				Purified	150 µg	610228

Human Adhesion Molecules - Catenins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
α-Catenin	C, Hu, Ms, Rat	5	Mouse IgG_1	Purified	50 µg	610193
				Purified	150 µg	610194
β-Catenin	C, Hu, Ms, Rat	14	Mouse IgG_1	Purified	50 µg	610153
				Purified	150 µg	610154
				FITC	50 µg	610155
				FITC	150 µg	610156
				HRP	50 µg	610157
				HRP	150 µg	610158
				TRITC	50 µg	610159
				TRITC	150 µg	610160
γ-Catenin	C, Hu, Ms, Rat	15	Mouse IgG_{2a}	Purified	50 µg	610253
				Purified	150 µg	610254
pp120	C, Hu, Ms, Rat	98	Mouse IgG_1	Purified	50 µg	610133
				Purified	150 µg	610134
				FITC	50 µg	610135
				FITC	150 µg	610136
				TRITC	50 µg	610137
				TRITC	150 µg	610138

Human Adhesion Molecules - ECM-Related

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
ADAM9	Hu, Ms, Rat	15	Mouse IgG_1	Purified	50 µg	610750
				Purified	150 µg	610751
Collagen VII α	Hu	23	Mouse IgG_1	Purified	50 µg	611606
				Purified	150 µg	611607
Fibronectin	C, Hu, Ms, Rat	10	Mouse IgG_1	Purified	50 µg	610077
				Purified	150 µg	610078
	Hu	2B6-D4	Mouse IgG_1, κ	Purified	0.1 mg	555867
Kalinin B1	Hu	17	Mouse IgG_1	Purified	50 µg	610423
				Purified	150 µg	610424
Laminin B2	Hu, Rat	22	Mouse IgG_1	Purified	50 µg	610722
				Purified	150 µg	610723
PAI-1	C, Hu, Ms, Rat	41	Mouse IgG_1	Purified	50 µg	612024
				Purified	150 µg	612025

Human Adhesion Molecules - Focal Adhesionion

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
FAK	C, Hu, Ms, Rat	77	Mouse IgG_1	Purified	50 µg	610087
				Purified	150 µg	610088
Phospho-FAK (Y397)	Hu, Ms	14	Mouse IgG_1	Purified	50 µg	611722
				Purified	150 µg	611723
Phospho-FAK (Y397)	Hu, Ms	18	Mouse IgG_1	Purified	50 µg	611806
				Purified	150 µg	611807
Hic-5	Hu, Ms, Rat	34	Mouse IgG_1	Purified	50 µg	611164
				Purified	150 µg	611165
p95PKL	Hu, Ms, Rat	13	Mouse IgG_1	Purified	50 µg	611388
				Purified	150 µg	611389
p130 Cas	C, Hu, Ms, Rat	21	Mouse IgG_1	Purified	50 µg	610271
				Purified	150 µg	610272
Paxillin	C, Hu, Ms, Rat	165	Mouse IgG_1	Purified	50 µg	610619
				Purified	150 µg	610620
	C, Hu, Ms, Rat	177	Mouse IgG_1	Purified	50 µg	610568
				Purified	150 µg	610569
	C, Hu, Ms, Rat	349	Mouse IgG_1	Purified	50 µg	610051
				Purified	150 µg	610052
				FITC	50 µg	610053
				FITC	150 µg	610054
				TRITC	50 µg	610055
				TRITC	150 µg	610056
Phospho-Paxillin (Y118)	Hu, Ms	30	Mouse IgG_1	Purified	50 µg	611724
				Purified	150 µg	611725
PYK2	C, Hu, Ms, Rat, Frog	11	Mouse IgG_1	Purified	50 µg	610548
				Purified	150 µg	610549
VASP	Hu	43	Mouse IgG_1	Purified	50 µg	610447
				Purified	150 µg	610448

Human Adhesion Molecules - Zonula Adherens

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
Desmoglein	C, Hu, Ms, Rat	62	Mouse IgG_1	Purified	50 µg	610273
				Purified	150 µg	610274
Occludin	Hu, Ms, Rat	19	Mouse IgG_1	Purified	50 µg	611090
				Purified	150 µg	611091
Symplekin	C, Hu, Ms, Rat	25	Mouse IgG_1	Purified	50 µg	610644
				Purified	150 µg	610645
ZO-1	Hu	1	Mouse IgG_1	Purified	50 µg	610966
				Purified	150 µg	610967

Human Adhesion Molecules - Other

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD34	Hu	563	Mouse IgG_1, κ	Purified	0.1 mg	550760
				PE	100 tests	550761
	Hu	581	Mouse IgG_1, κ	Purified	0.1 mg	555820
				FITC	100 tests	555821
				PE	100 tests	555822
				Cy-Chrome	100 tests	555823
				APC	100 tests	555824
CD36	Hu	25	Mouse IgG_1	Purified	50 µg	610882
				Purified	150 µg	610883
	Hu	CB38 (NL07)	Mouse IgM, κ	Purified	0.1 mg	555453
			(Sprague-Dawley)	FITC	100 tests	555454
				PE	100 tests	555455
CD38	Hu	22	Mouse IgG_1	Purified	50 µg	611114
				Purified	150 µg	611115
CD42a	Hu	ALMA.16	Mouse IgG_1	Purified	0.1 mg	558816
			(Sprague-Dawley)	FITC	100 tests	558818
				PE	100 tests	558819
CD42b	Hu	HIP1	Mouse IgG_1, κ	NA/LE	0.5 mg	555470
			(Sprague-Dawley)	Purified	0.1 mg	555471
				FITC	100 tests	555472
				PE	100 tests	555473
CD43	Hu	1G10	Mouse IgG_1, κ	NA/LE	0.5 mg	559863
				Purified	0.1 mg	555474
				FITC	100 tests	555475
CD44	Hu	G44-26	Mouse IgG_{2b}, κ	Purified	0.1 mg	555476
				Biotin	100 tests	555477
				FITC	100 tests	555478
				PE	100 tests	555479
				APC	100 tests	559942
		L178	Mouse IgG_1	Purified	0.2 mg	559046
CD105	Hu	35	Mouse IgG_1	Purified	50 µg	611314
				Purified	150 µg	611315
	Hu	266	Mouse IgG_1, κ	Purified	0.1 mg	555690
			(Sprague-Dawley)			
CD107a	Hu	H4A3	Mouse IgG_1, κ	Purified	0.1 mg	555798
			(Sprague-Dawley)	Biotin	100 tests	555799
				FITC	100 tests	555800
				PE	100 tests	555801
				Cy-Chrome	100 tests	555802
CD107b	Hu	H4B4	Mouse IgG_1, κ	Purified	0.1 mg	555803
			(Sprague-Dawley)	FITC	100 tests	555804
CD134	Hu	ACT35	Mouse IgG_1, κ	Purified	0.1 mg	555836
				FITC	100 tests	555837
				PE	100 tests	555838
CD144	Hu	55-7H1	Mouse IgG_1, κ	Purified	0.1 mg	555661
CD162	Hu	KPL-1	Mouse IgG_1	NA/LE	0.5 mg	556052
				Purified	0.1 mg	556053
				PE	100 tests	556055
Attractin	Hu	14	Mouse IgG_1	Purified	50 µg	611530
				Purified	150 µg	611531

Human Adhesion Molecules - Other (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CLA	Hu	HECA-452	Rat IgM, κ	Purified	0.1 mg	555946
			(Sprague-Dawley)	Biotin	100 tests	559950
				FITC	100 tests	555947
b-Dystroglycan	Hu	56	Mouse IgG_{2a}	Purified	50 µg	612090
				Purified	150 µg	612091
Fibrinogen	Hu	2C2-G7	Mouse IgG_1, κ	Purified	0.1 mg	555866
LR11	Hu, Ms, Rat	48	Mouse IgG_{2a}	Purified	50 µg	611860
				Purified	150 µg	611861
Mena	C, Hu, Ms, Rat	21	Mouse IgA	Purified	50 µg	610692
				Purified	150 µg	610693
Neurexin I	Hu, Ms, Rat	17	Mouse IgG_1	Purified	50 µg	611882
				Purified	150 µg	611883
Nexilin	Hu, Ms, Rat	25	Mouse IgG_1	Purified	50 µg	611556
				Purified	150 µg	611557
Ninjurin	Hu, Ms, Rat	50	Mouse IgG_{2a}	Purified	50 µg	610776
				Purified	150 µg	610777
Plakophilin 2a	Hu	28	Mouse IgG_1	Purified	50 µg	610788
				Purified	150 µg	610789

Human Adhesion Molecules - MMPs & Inhibitors

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
MMP Inhibitor				Peptide	0.5 mg	556455
MMP-1	Hu	III12b	Mouse (BALB/c) IgG_1	Purified	0.1 mg	550941
MMP-2	Hu	A-Gel VC2	Mouse (BALB/c) IgG_1	Purified	0.1 mg	550892
MMP-3	Hu	SL-1 IID4	Mouse (BALB/c) IgG_{2b}	Purified	0.1 mg	550893
				Purified	1.0 ml	551117
MMP-9	Hu	IIA5	Mouse (BALB/c) IgG_1	Purified	0.1 mg	550942
				Purified	1.0 ml	551005
MMP-10	Hu	IVC5	Mouse IgG_{2b}, κ	Purified	1.0 ml	551118
MMP-11	Hu	SL3.05	Mouse IgG_1, κ	Purified	1.0 ml	551006
TIMP-1	Hu	102D1	Mouse (BALB/c) IgG_1	Purified	0.1 mg	550940
				Purified	1.0 ml	551007
TIMP-2	Hu	T2-101	Mouse (BALB/c) IgG_1	Purified	0.1 mg	550898

Mouse Adhesion Molecules - Selectins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD62E	Ms	10E9.6	Rat (Lewis) IgG_{2a}, κ	NA/LE	0.5 mg	553748
				Purified	0.5 mg	553749
				Purified	1.0 ml	550290
				Biotin	0.5 mg	553750
				PE	0.2 mg	553751
CD62L	Ms	MEL-14	Rat (Fischer) IgG_{2a}, κ	NA/LE	0.5 mg	553147
				Purified	0.5 mg	553148
				Purified	1.0 ml	550288
				Biotin	0.5 mg	553149
				FITC	0.5 mg	553150
				PE	0.2 mg	553151
				APC	0.1 mg	553152

Mouse Adhesion Molecules - Selectins (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD62P	Ms	Polyclonal	Rabbit IgG	NA/LE	SO	
				Purified	0.1 mg	553716
		RB40.34	Rat (Lewis) IgG_1, l	NA/LE	0.5 mg	553741
				Purified	0.5 mg	553742
				Purified	1.0 ml	550289
				Biotin	0.5 mg	553743
				FITC	0.5 mg	553744

Mouse Adhesion Molecules - Ig Superfamily

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD2	Ms	RM2-5	Rat (SD) IgG_{2b}, l	NA/LE	SO	
				Purified	0.5 mg	553109
				Biotin	0.5 mg	553110
				FITC	0.5 mg	553111
				PE	0.2 mg	553112
CD22.2	Ms	Cy34.1	Mouse (DBA/1) IgG_1, κ	Purified	0.1 mg	557473
				Biotin	0.5 mg	553382
				FITC	0.1 mg	557474
				PE	0.2 mg	553384
CD31	Ms	390	Rat (Lewis) IgG_{2a}, κ	NA/LE	0.5 mg	553708
				Purified	0.1 mg	558736
				Biotin	0.1 mg	558737
				FITC	0.1 mg	558738
		MEC 13.3	Rat IgG_{2a}, κ	NA/LE	0.5 mg	553369
			(LEW/Cr1BR)	Purified	0.1 mg	557355
				Purified	0.5 mg	553370
				Purified	1.0 ml	550274
				Biotin	0.5 mg	553371
				FITC	0.5 mg	553372
				PE	0.2 mg	553373
				APC	0.1 mg	551262
CD48	Ms	HM48-1	Armenian	NA/LE	0.5 mg	553682
			Hamster IgG_1, λ	Purified	0.1 mg	557483
				FITC	0.1 mg	557484
				PE	0.1 mg	557485
CD54	Ms	3.00E+02	Armenian	NA/LE	0.5 mg	553249
			Hamster IgG_1, κ	Purified	0.5 mg	553250
				Purified	1.0 ml	550287
				Biotin	0.5 mg	553251
				FITC	0.5 mg	553252
				PE	0.2 mg	553253
CD56	Ms	12F11	Rat (SD) IgG_{2a}	Purified	0.1 mg	556323
		12F8	Rat (SD) IgM	Purified	0.1 mg	556325
		N-CAM 13	Mouse (SD) IgG_{2a}	Purified	0.1 mg	556324
CD102	Ms	3C4 (mIC2/4)	Rat (Lewis) IgG_{2a}, κ	NA/LE	0.5 mg	553325
				Purified	0.5 mg	553326
				Purified	1.0 ml	550544
				Biotin	0.1 mg	557441
				FITC	0.1 mg	557444

Mouse Adhesion Molecules - Ig Superfamily (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD106	Ms	429 (MVCAM.A)	Rat (Lewis) IgG_{2a}, κ	NA/LE	0.5 mg	553329
				Purified	0.5 mg	553330
				Purified	1.0 ml	556324
				Biotin	0.5 mg	553331
				FITC	0.5 mg	553332
Contactin	Hu, Ms, Rat	41	Mouse IgG_1	Purified	50 µg	610578
				Purified	150 µg	610579
L1	C, Hu, Ms, Rat	17	Mouse IgG_{2b}	Purified	50 µg	610302
				Purified	150 µg	610303
LAR	Hu, Ms, Rat	7	Mouse IgG_{2a}	Purified	50 µg	610350
				Purified	150 µg	610351
RNCAM	Hu, Ms, Rat	44	Mouse IgG_1	Purified	50 µg	611092
				Purified	150 µg	611093
RPTP β	Hu, Ms, Rat	12	Mouse IgG_1	Purified	50 µg	610179
				Purified	150 µg	610180

Mouse Adhesion Molecules - Integrins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD11a	Ms	2D7	Rat IgG_{2a}, κ	NA/LE	SO	
				Purified	0.5 mg	553118
				Biotin	0.1 mg	557365
				FITC	0.5 mg	553120
				PE	0.1 mg	553121
		M17/4	Rat IgG_{2a}, κ	NA/LE	0.5 mg	553337
			(Wistar/Furth)	Purified	0.1 mg	557453
				Purified	1.0 ml	550528
				Biotin	0.1 mg	557455
				FITC	0.5 mg	553340
CD11b	Ms	M1/70	Rat (DA) IgG_{2b}, κ	NA/LE	0.5 mg	553307
				Purified	0.1 mg	557394
				Purified	0.5 mg	553308
				Purified	1.0 ml	550282
				Biotin	0.5 mg	553309
				FITC	0.1 mg	557396
				FITC	0.5 mg	553310
				PE	0.1 mg	557397
				PE	0.2 mg	553311
				APC	0.1 mg	553312
				PerCP-Cy5.5	0.1 mg	550993
CD11c	Ms	HL3	Armenian	Purified	0.5 mg	553799
			Hamster IgG_1, λ	Purified	1.0 ml	550283
				Biotin	0.5 mg	553800
				FITC	0.1 mg	557400
				FITC	0.5 mg	553801
				PE	0.1 mg	557401
				PE	0.2 mg	553802
				APC	0.1 mg	550261

Mouse Adhesion Molecules - Integrins (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD18	Ms	C71/16	Rat (Lewis) IgG_{2a}, κ	Purified	0.1 mg	557438
				Biotin	0.1 mg	557439
				FITC	0.5 mg	553292
				PE	0.1 mg	553293
		GAME-46	Rat IgG_1, κ	NA/LE	0.5 mg	555280
			(Sprague-Dawley)	Purified	0.1 mg	557440
		M18/2	Rat IgG_{2a}, κ	NA/LE	0.5 mg	553341
				Purified	0.1 mg	557437
CD29	Ms	9EG7	Rat IgG_{2a}, κ	NA/LE	SO	
				Purified	0.5 mg	553715
				Purified	1.0 ml	550531
	Ms, Rat	Ha2/5	Armenian	NA/LE	0.5 mg	555002
			Hamster IgM, κ	Purified	0.5 mg	555003
				Biotin	0.5 mg	555004
				FITC	0.5 mg	555005
				Purified	0.5 mg	553837
	Ms	KMI6	Rat (LOU/MN) IgG_{2a}, κ	NA/LE	SO	
				Purified	0.1 mg	558741
CD41	Ms	MWReg30	Rat IgG_1, κ	Purified	0.5 mg	553847
				FITC	0.5 mg	553848
CD49a	Ms, Rat	Ha31/8	Armenian	NA/LE	0.5 mg	555000
			Hamster IgG_2, λ	Purified	0.5 mg	555001
				Purified	1.0 ml	550568
CD49b	Ms, Rat	Ha1/29	Armenian	NA/LE	SO	
			Hamster IgG, λ	Purified	0.1 mg	559987
				Purified	0.5 mg	554998
				FITC	0.5 mg	554999
	Ms	HMa2	Armenian	NA/LE	0.5 mg	557017
			Hamster IgG_1, κ	Purified	0.5 mg	553819
				PE	0.1 mg	558759
CD49c	Dog, Ms, Rat	42	Mouse IgG_1	Purified	50 µg	611044
				Purified	150 µg	611045
CD49d	Ms	9C10 (MFR4.B)	Rat (Lewis) IgG_{2a}, κ	NA/LE	0.5 mg	553313
				Purified	0.5 mg	553314
				Biotin	0.1 mg	557419
				PE	0.1 mg	557420
		R1-2	Rat (Fischer) IgG_{2b}, κ	NA/LE	0.5 mg	553153
				Purified	0.5 mg	553154
				Biotin	0.1 mg	557406
				FITC	0.5 mg	553156
				PE	0.2 mg	553157
		SG31	Rat IgG_{2a}, κ	NA/LE	0.5 mg	559131
				Purified	0.1 mg	559132
				PE	0.1 mg	559134
CD49e	Ms	5H10-27	Rat (Lewis) IgG_{2a}, κ	NA/LE	0.5 mg	553318
				Purified	0.5 mg	553319
				Biotin	0.1 mg	557446
				PE	0.1 mg	557447
	Ms, Rat	HMa5-1	Armenian	NA/LE	SO	
			Hamster IgG_1, κ	Purified	0.5 mg	553350
CD49f	Hu, Ms	GoH3	Rat IgG_{2a}, κ	Purified	0.1 mg	555734
			(Sprague-Dawley)	FITC	100 tests	555735
				PE	100 tests	555736

Mouse Adhesion Molecules - Integrins (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD51	Dog, Hu, Ms, Rat	21	Mouse IgG_1	Purified	50 µg	611012
				Purified	150 µg	611013
	Ms	H9.2B8	Armenian Hamster IgG_3, λ	NA/LE	0.5 mg	553241
				Purified	SO	
		RMV-7	Rat IgG_1, κ (Sprague-Dawley)	NA/LE	0.5 mg	550023
				Purified	0.1 mg	550024
				Biotin	0.1 mg	551380
				PE	0.1 mg	551187
CD61	Ms, Rat	2C9.G2	Armenian Hamster IgG_1, κ	NA/LE	0.5 mg	553343
				Purified	0.5 mg	553344
				Purified	1.0 ml	550541
				Biotin	0.5 mg	553345
				FITC	0.5 mg	553346
				PE	0.2 mg	553347
CD103	Ms	M290	Rat (Lou/c) IgG_{2a}, κ	NA/LE	0.5 mg	553699
				Purified	0.1 mg	557492
				Biotin	0.1 mg	557493
				FITC	0.1 mg	557494
				PE	0.1 mg	557495
		2.00E+07	Armenian Hamster IgG_2, κ	NA/LE	SO	
				Purified	0.1 mg	557475
				Biotin	0.1 mg	557476
				FITC	0.1 mg	557521
CD104	Ms	346-11A	Rat IgG_{2a}, κ (Fischer 344)	Purified	0.5 mg	553745
				Purified	1.0 ml	550545
Integrin β1	C, Hu, Ms, Rat	18	Mouse IgG_1	Purified	50 µg	610467
				Purified	150 µg	610468
Integrin β7	Ms	FIB27	Rat (Fischer) IgG_{2a}, κ	NA/LE	0.5 mg	553812
				Purified	0.1 mg	557499
		M293	Rat (Lou/c) IgG_{2a}, κ	Purified	0.5 mg	553704
				Biotin	0.1 mg	557496
				FITC	0.1 mg	557497
				PE	0.1 mg	557498
LPAM-1	Ms	DATK32	Rat (Fischer) IgG_{2a}, κ	NA/LE	0.5 mg	553809
				Purified	0.1 mg	558767
				PE	0.2 mg	553811

Mouse Adhesion Molecules - Cadherins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
E-Cadherin	Hu, Ms, Rat	36	Mouse IgG_{2a}	Purified	50 µg	610181
				Purified	150 µg	610182
M-Cadherin	Ms, Rat	5	Mouse IgG_{2a}	Purified	50 µg	611100
				Purified	150 µg	611101
N-Cadherin	C, Hu, Ms, Rat	32	Mouse IgG_1	Purified	50 µg	610920
				Purified	150 µg	610921
R-Cadherin	Ms, Rat	48	Mouse IgG_1	Purified	50 µg	610414
				Purified	150 µg	610415

Mouse Adhesion Molecules - Catenins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
α-Catenin	C, Hu, Ms, Rat	5	Mouse IgG_1	Purified	50 µg	610193
				Purified	150 µg	610194
β-Catenin	C, Hu, Ms, Rat	14	Mouse IgG_1	Purified	50 µg	610153
				Purified	150 µg	610154
				FITC	50 µg	610155
				FITC	150 µg	610156
				HRP	50 µg	610157
				HRP	150 µg	610158
				TRITC	50 µg	610159
				TRITC	150 µg	610160
δ-Catenin	Ms, Rat	30	Mouse IgG_1	Purified	50 µg	611536
				Purified	150 µg	611537
γ-Catenin	C, Hu, Ms, Rat	15	Mouse IgG_{2a}	Purified	50 µg	610253
				Purified	150 µg	610254
pp120	C, Hu, Ms, Rat	98	Mouse IgG_1	Purified	50 µg	610133
				Purified	150 µg	610134
				FITC	50 µg	610135
				FITC	150 µg	610136
				TRITC	50 µg	610137
				TRITC	150 µg	610138

Mouse Adhesion Molecules - ECM-Related

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
ADAM9	Hu, Ms, Rat	15	Mouse IgG_1	Purified	50 µg	610750
				Purified	150 µg	610751
Brevican	Ms, Rat	2	Mouse IgG_1	Purified	50 µg	610894
				Purified	150 µg	610895
Fibronectin	C, Hu, Ms, Rat	10	Mouse IgG_1	Purified	50 µg	610077
				Purified	150 µg	610078
PAI-1	C, Hu, Ms, Rat	41	Mouse IgG_1	Purified	50 µg	612024
				Purified	150 µg	612025
Thrombospondin-2	Ms	4	Mouse IgG_1	Purified	50 µg	611150
				Purified	150 µg	611151

Mouse Adhesion Molecules - Focal Adhesion

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
FAK	C, Hu, Ms, Rat	77	Mouse IgG_1	Purified	50 µg	610087
				Purified	150 µg	610088
Phospho-FAK (Y397)	Hu, Ms	14	Mouse IgG_1	Purified	50 µg	611722
				Purified	150 µg	611723
Phospho-FAK (Y397)	Hu, Ms	18	Mouse IgG_1	Purified	50 µg	611806
				Purified	150 µg	611807
Hic-5	Hu, Ms, Rat	34	Mouse IgG_1	Purified	50 µg	611164
				Purified	150 µg	611165
p95PKL	Hu, Ms, Rat	13	Mouse IgG_1	Purified	50 µg	611388
				Purified	150 µg	611389

Mouse Adhesion Molecules - Focal Adhesion (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
p130Cas	C, Hu, Ms, Rat	21	Mouse IgG_1	Purified	50 µg	610271
				Purified	150 µg	610272
Paxillin	C, Hu, Ms, Rat	165	Mouse IgG_1	Purified	50 µg	610619
				Purified	150 µg	610620
	C, Hu, Ms, Rat	177	Mouse IgG_1	Purified	50 µg	610568
				Purified	150 µg	610569
	C, Hu, Ms, Rat	349	Mouse IgG_1	Purified	50 µg	610051
				Purified	150 µg	610052
				FITC	50 µg	610053
				FITC	150 µg	610054
				TRITC	50 µg	610055
				TRITC	150 µg	610056
Phospho-Paxillin (Y118)	Hu, Ms	30	Mouse IgG_1	Purified	50 µg	611724
				Purified	150 µg	611725
PYK2	C, Hu, Ms, Rat, Frog	11	Mouse IgG_1	Purified	50 µg	610548
				Purified	150 µg	610549

Mouse Adhesion Molecules - Zonula Adherens

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
Desmoglein	C, Hu, Ms, Rat	62	Mouse IgG_1	Purified	50 µg	610273
				Purified	150 µg	610274
Occludin	Hu, Ms, Rat	19	Mouse IgG_1	Purified	50 µg	611090
				Purified	150 µg	611091
Symplekin	C, Hu, Ms, Rat	25	Mouse IgG_1	Purified	50 µg	610644
				Purified	150 µg	610645
ZO-2	Ms, Rat	3	Mouse IgG_1	Purified	50 µg	611560
				Purified	150 µg	611561

Mouse Adhesion Molecules - Other

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD43	Ms	1B11	Rat (Wistar) IgG_{2a}, κ	Purified	0.1 mg	558760
				FITC	0.1 mg	558761
				PE	0.1 mg	558762
CD43	Ms	S7	Rat IgG_{2a}, κ	NA/LE	SO	
			[(DA x LOU) IF, Hybrid)]	Purified	0.5 mg	553268
				Biotin	0.5 mg	553269
				FITC	0.5 mg	553270
				PE	0.2 mg	553271

Mouse Adhesion Molecules - Other (*continued*)

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD44	Ms	IM7	Rat IgG_{2b}, κ	NA/LE	0.5 mg	553130
				Purified	0.5 mg	553131
				Purified	1.0 ml	550538
				Biotin	0.5 mg	553132
				FITC	0.5 mg	553133
				PE	0.1 mg	553134
				Cy-Chrome	0.1 mg	553135
				APC	0.1 mg	559250
		KM114	Rat (LOU/MN) IgG_1, κ	NA/LE	0.5 mg	553724
				Purified	0.1 mg	558739
CD44H	Ms	TM-1	Armenian Hamster IgG_3, λ	Purified	0.1 mg	551453
CD105	Ms	MJ7/18	Rat IgG_{2a}, κ	Purified	1.0 ml	550546
CD107a	Ms	1D4B	Rat IgG_{2a}, κ	Purified	0.5 mg	553792
			(Sprague-Dawley)	FITC	0.5 mg	553793
CD107b	Ms	ABL-93	RatIgG_{2a}, κ	Purified	0.1 mg	558756
			(Sprague-Dawley)	FITC	0.1 mg	558758
CD138	Ms	281-2	Rat (Fischer) IgG_{2a}, κ	Purified	0.5 mg	553712
				Biotin	0.5 mg	553713
				PE	0.2 mg	553714
CD144	Ms	11D4.1	Rat (Lewis) IgG_{2a}, κ	NA/LE	SO	
				Purified	0.5 mg	555289
				Purified	1.0 ml	550548
CD162	Ms	2PH1	Rat (Lewis) IgG_1, κ	NA/LE	0.5 mg	555304
				Purified	0.1 mg	555294
				Purified	0.5 mg	555305
				PE	0.2 mg	555306
LR11	Hu, Ms, Rat	48	Mouse IgG_{2a}	Purified	50 µg	611860
				Purified	150 µg	611861
MAdCAM-1	Ms	MECA-367	Rat (Wistar) IgG_{2a}, κ	NA/LE	0.5 mg	553805
				Purified	0.5 mg	553806
				Purified	1.0 ml	550556
		MECA-89	Rat (Wistar) IgG_{2a}, κ	Purified	1.0 ml	550555
				Biotin	0.5 mg	553808
Mena	C, Hu, Ms, Rat	21	Mouse IgA	Purified	50 µg	610692
				Purified	150 µg	610693
Neurexin I	Hu, Ms, Rat	17	Mouse IgG_1	Purified	50 µg	611882
				Purified	150 µg	611883
Nexilin	Hu, Ms, Rat	25	Mouse IgG_1	Purified	50 µg	611556
				Purified	150 µg	611557
				Purified	150 µg	610777
PNAd Carbohydrate Epitope	Ms	MECA-79	Rat (Wistar) IgM, κ	NA/LE	SO	
				Purified	0.5 mg	553863
				Purified	1.0 ml	550564
VAP-1	Dog, Ms, Rat	54	Mouse IgG_1	Purified	50 µg	611190l
				Purified	150 µg	611191

Rat Adhesion Molecules - Selectins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD62L	Rat	HRL1	Armenian Hamster IgG_2, λ	NA/LE	SO	
				Purified	0.1 mg	559988
				Biotin	0.1 mg	559989
				FITC	0.5 mg	554963
				PE	0.1 mg	551398
		HRL2	Armenian Hamster IgG_1, κ	Purified	0.1 mg	559990
CD62P	Ms	Polyclonal	Rabbit IgG	NA/LE	SO	
				Purified	0.1 mg	553716

Rat Adhesion Molecules - Ig Superfamily

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD2	Rat	OX-34	Mouse IgG_{2a}, κ	NA/LE	SO	
				Purified	0.5 mg	554826
				FITC	0.5 mg	554827
CD31	Rat	TLD-3A12	Mouse (BALB/c) IgG_1, κ	NA/LE	0.5 mg	555024
				Purified	0.5 mg	555025
				Purified	1.0 ml	550300
				Biotin	0.5 mg	555026
				PE	0.1 mg	555027
CD54	Rat	1A29	Mouse (BALB/c) IgG_1, κ	NA/LE	0.5 mg	554966
				Purified	0.5 mg	554967
				Purified	1.0 ml	550302
				Biotin	0.5 mg	554968
				FITC	0.5 mg	554969
				PE	0.2 mg	554970
CD106	Rat	MR106	Mouse IgG_1, κ	Purified	0.5 mg	559165
				PE	0.1 mg	559229
Contactin	Hu, Ms, Rat	41	Mouse IgG_1	Purified	50 µg	610578
				Purified	150 µg	610579
L1	C, Hu, Ms, Rat	17	Mouse IgG_{2b}	Purified	50 µg	610302
				Purified	150 µg	610303
LAR	Hu, Ms, Rat	7	Mouse IgG_{2a}	Purified	50 µg	610350
				Purified	150 µg	610351
RNCAM	Hu, Ms, Rat	44	Mouse IgG_1	Purified	50 µg	611092
				Purified	150 µg	611093
RPTP β	Hu, Ms, Rat	12	Mouse IgG_1	Purified	50 µg	610179
				Purified	150 µg	610180

Rat Adhesion Molecules - Integrins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD11a	Rat	WT.1	Mouse (BALB/c) IgG_{2a}, κ	NA/LE	SO	
				Purified	0.1 mg	559979
				FITC	0.5 mg	554985
				PE	0.1 mg	550972
CD11b	Rat	WT.5	Mouse (BALB/c) IgA, κ	NA/LE	SO	
				Purified	0.5 mg	554980
				Biotin	0.5 mg	554981
				FITC	0.5 mg	554982
CD11b/c	Rat	OX-42	Mouse IgG_{2a}, κ	NA/LE	0.5 mg	554858
			(BALB/c)	Purified	0.5 mg	554859
				Purified	1.0 ml	550299
				FITC	0.5 mg	554861
				PE	0.2 mg	554862
CD18	Rat	WT.3	Mouse (BALB/c) IgG_1, κ	NA/LE	0.5 mg	554976
				Purified	0.5 mg	554977
				FITC	0.5 mg	554979
CD29	Ms, Rat	Ha2/5	Armenian	NA/LE	0.5 mg	555002
			Hamster IgM, κ	Purified	0.5 mg	555003
				Biotin	0.5 mg	555004
				FITC	0.5 mg	555005
	Ms	HMb1-1	Armenian	NA/LE	SO	
			Hamster IgG_2, λ	Purified	1.0 ml	550530
CD49a	Ms, Rat	Ha31/8	Armenian	NA/LE	0.5 mg	555000
			Hamster IgG_2, λ	Purified	0.5 mg	555001
				Purified	1.0 ml	550568
CD49b	Ms, Rat	Ha1/29	Armenian	NA/LE	SO	
			Hamster IgG_2, λ	Purified	0.1 mg	559987
				Purified	0.5 mg	554998
				FITC	0.5 mg	554999
CD49c	Dog, Ms, Rat	42	Mouse IgG_1	Purified	50 µg	611044
				Purified	150 µg	611045
CD49d	Rat	MRa4-1	Mouse IgG_{2a}, κ	NA/LE	SO	
			(BALB/c)	Purified	0.5 mg	553348
				FITC	0.1 mg	557457
CD49e	Ms, Rat	HMa5-1	Armenian	NA/LE	SO	
			Hamster IgG_1, κ	Purified	0.5 mg	553350
CD51	Dog, Hu, Ms, Rat	21	Mouse IgG_1	Purified	50 µg	611012
				Purified	150 µg	611013
CD61	Rat	F11	Mouse IgG_1, κ	NA/LE	0.5 mg	554950
			(BALB/c)	Purified	0.5 mg	554951
	Ms, Rat	2C9.G2	Armenian	NA/LE	0.5 mg	553343
			Hamster IgG_1, κ	Purified	0.5 mg	553344
				Purified	1.0 ml	550541
				Biotin	0.5 mg	553345
				FITC	0.5 mg	553346
				PE	0.2 mg	553347
Integrin β1	C, Hu, Ms, Rat	18	Mouse IgG_1	Purified	50 µg	610467
				Purified	150 µg	610468

Rat Adhesion Molecules - Cadherins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
E-Cadherin	Hu, Rat	34	Mouse IgG_{2b}	Purified	50 µg	610404
				Purified	150 µg	610405
	Hu, Ms, Rat	36	Mouse IgG_{2a}	Purified	50 µg	610181
				Purified	150 µg	610182
M-Cadherin	Ms, Rat	5	Mouse IgG_{2a}	Purified	50 µg	611100
				Purified	150 µg	611101
N-Cadherin	C, Hu, Ms, Rat	32	Mouse IgG_1	Purified	50 µg	610920
				Purified	150 µg	610921
P-Cadherin	Hu, Rat	56	Mouse IgG_1	Purified	50 µg	610227
				Purified	150 µg	610228
R-Cadherin	Ms, Rat	48	Mouse IgG_1	Purified	50 µg	610414
				Purified	150 µg	610415l

Rat Adhesion Molecules - Catenins

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
α-Catenin	C, Hu, Ms, Rat	5	Mouse IgG_1	Purified	50 µg	610193
				Purified	150 µg	610194
β-Catenin	C, Hu, Ms, Rat	14	Mouse IgG_1	Purified	50 µg	610153
				Purified	150 µg	610154
				FITC	50 µg	610155
				FITC	150 µg	610156
				HRP	50 µg	610157
				HRP	150 µg	610158
				TRITC	50 µg	610159
				TRITC	150 µg	610160
δ-Catenin	Ms, Rat	30	Mouse IgG_1	Purified	50 µg	611536
				Purified	150 µg	611537
γ-Catenin	C, Hu, Ms, Rat	15	Mouse IgG_{2a}	Purified	50 µg	610253
				Purified	150 µg	610254
pp120	C, Hu, Ms, Rat	98	Mouse IgG_1	Purified	50 µg	610133
				Purified	150 µg	610134
				FITC	50 µg	610135
				FITC	150 µg	610136
				TRITC	50 µg	610137
				TRITC	150 µg	610138

Rat Adhesion Molecules - ECM-Related

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
ADAM9	Hu, Ms, Rat	15	Mouse IgG_1	Purified	50 µg	610750
				Purified	150 µg	610751
Brevican	Ms, Rat	2	Mouse IgG_1	Purified	50 µg	610894
				Purified	150 µg	610895
Fibronectin	C, Hu, Ms, Rat	10	Mouse IgG_1	Purified	50 µg	610077
				Purified	150 µg	610078
Laminin B2	Hu, Rat	22	Mouse IgG_1	Purified	50 µg	610722
				Purified	150 µg	610723
Neuroglycan C	Rat	1	Mouse IgG_1	Purified	50 µg	610986
				Purified	150 µg	610987
PAI-1	C, Hu, Ms, Rat	41	Mouse IgG_1	Purified	50 µg	612024
				Purified	150 µg	612025
Thrombospondin-1	Rat	20	Mouse IgG_1	Purified	50 µg	610916
				Purified	150 µg	610917

Rat Adhesion Molecules - Focal Adhesion

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
FAK	C, Hu, Ms, Rat	77	Mouse IgG_1	Purified	50 µg	610087
				Purified	150 µg	610088
Hic-5	Hu, Ms, Rat	34	Mouse IgG_1	Purified	50 µg	611164
				Purified	150 µg	611165
p95PKL	Hu, Ms, Rat	13	Mouse IgG_1	Purified	50 µg	611388
				Purified	150 µg	611389
p130 Cas	C, Hu, Ms, Rat	21	Mouse IgG_1	Purified	50 µg	610271
				Purified	150 µg	610272
Paxillin	C, Hu, Ms, Rat	165	Mouse IgG_1	Purified	50 µg	610619
				Purified	150 µg	610620
	C, Hu, Ms, Rat	177	Mouse IgG_1	Purified	50 µg	610568
				Purified	150 µg	610569
	C, Hu, Ms, Rat	349	Mouse IgG_1	Purified	50 µg	610051
				Purified	150 µg	610052
				FITC	50 µg	610053
				FITC	150 µg	610054
				TRITC	50 µg	610055
				TRITC	150 µg	610056
PYK2	C, Hu, Ms, Rat, Frog	11	Mouse IgG_1	Purified	50 µg	610548
				Purified	150 µg	610549

Rat Adhesion Molecules - Zonula Adherens

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
Desmoglein	C, Hu, Ms, Rat	62	Mouse IgG_1	Purified	50 µg	610273
				Purified	150 µg	610274
Occludin	Hu, Ms, Rat	19	Mouse IgG_1	Purified	50 µg	611090
				Purified	150 µg	611091
Symplekin	C, Hu, Ms, Rat	25	Mouse IgG_1	Purified	50 µg	610644
				Purified	150 µg	610645
ZO-2	Ms, Rat	3	Mouse IgG_1	Purified	50 µg	611560
				Purified	150 µg	611561

Rat Adhesion Molecules - Other

Description	Reactivity	Clone	Isotype	Format	Size	Cat. No.
CD43	Rat	HIS17	Mouse (BALB/c) IgG_1, κ	Purified	0.5 mg	554867
				Biotin	0.5 mg	554868
CD44H	Rat	OX-49	Mouse IgG_{2a}, κ	Purified	0.5 mg	554869
			(BALB/c)	FITC	0.1 mg	550974
CD98HC	Rat	30	Mouse IgG_1	Purified	50 µg	611516
				Purified	150 µg	611517
CD134	Rat	OX-40	Mouse IgG_{2b}, κ	NA/LE	SO	
			(BALB/c)	Purified	0.1 mg	559991
				Biotin	0.1 mg	550977
				FITC	0.5 mg	554848
LR11	Hu, Ms, Rat	48	Mouse IgG_{2a}	Purified	50 µg	611860
				Purified	150 µg	611861
Mena	C, Hu, Ms, Rat	21	Mouse IgA	Purified	50 µg	610692
				Purified	150 µg	610693
Neurexin I	Hu, Ms, Rat	17	Mouse IgG_1	Purified	50 µg	611882
				Purified	150 µg	611883
Nexilin	Hu, Ms, Rat	25	Mouse IgG_1	Purified	50 µg	611556
				Purified	150 µg	611557
Ninjurin	Hu, Ms, Rat	50	Mouse IgG_{2a}	Purified	50 µg	610776
				Purified	150 µg	610777
VAP-1	Dog, Ms, Rat	54	Mouse IgG_1	Purified	50 µg	611190
				Purified	150 µg	611191

Adhesion-Related Sampler Kits

Description	Reactivity	Format	Size	Cat. No.
Axon Guidance Sampler Kit		Kit	10 ug ea. ab	611660
Focal Adhesion Sampler Kit		Kit	10 ug ea. ab	611433
Integrins Sampler Kit		Kit	10 ug ea. ab	611435
Zonula Adherens Sampler Kit		Kit	10 ug ea. ab	611446

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