

Rat Immunology

From Genes to Proteins to Cells

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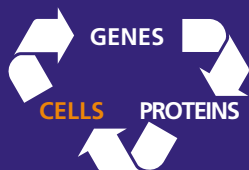


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Introduction

The rat is the second most frequently used animal, after the mouse, in the field of immunological research. Traditionally, rats have been used as experimental animals to study behavior, physiology, and pathology, including tumor biology, transplantation, and toxicology. Together with mice and guinea pigs, rats belong to the largest order of mammals, the Rodentia. The laboratory rat was developed from the brown rat, *Rattus norvegicus*, at the beginning of the twentieth century, and most laboratory rat strains originate from animals held at the Wistar Institute of Anatomy and Biology in Philadelphia, USA.

A large number of genetically well-defined inbred laboratory rat strains are now available for experimental research. In addition, a variety of congenic, mutant and recombinant rat strains of immunological interest have been developed in recent years, further expanding the possibilities of experimentation. These strains include nude (athymic) rats, rats that spontaneously develop diabetes, and pairs of rats congenic for MHC class I (RT1A) or II (RT1B and RT1D) haplotypes, immunoglobulin kappa light chain (Ig κ -1) allotypes or the leukocyte common alloantigen (RT7). BD PharMingen offers antibodies that recognize polymorphic and non-polymorphic epitopes of those alloantigen systems. (Please see the Rat MHC Haplotype chart on page 11.) Although rats are small animals, they are big enough to allow a wide variety of experimental manipulations. Their comparably larger size, than mice, offers unique possibilities for the application of microsurgical techniques, therefore rats are often

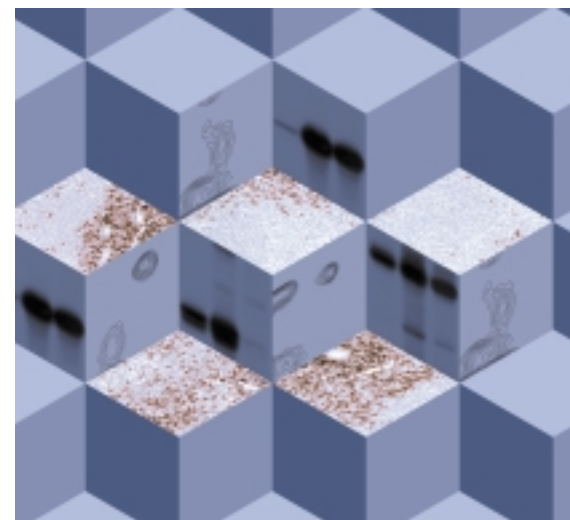
the species of premier choice for transplantation studies. For example, the recent technology to induce indefinite allograft survival by intrathymic injection of alloantigen was first developed in rats. Rats are also used frequently for the generation of hybridomas and monoclonal antibodies. There are many rat autoimmune disease models which develop spontaneously after immunization with appropriate antigens, after chemical treatments, or upon introduction of a transgene. Some examples of frequently used rat models of human autoimmune diseases are:

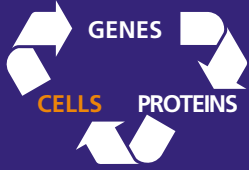
- **Insulin-dependent diabetes mellitus (IDDM).** Diabetes-prone bio-breeding (DP-BB) rats spontaneously develop autoimmune insulin-dependent diabetes and thyroiditis at very high frequency. Hyperglycemia develops when rats are between 50 and 90 days old and is associated with infiltration of the islets of Langerhans and selective destruction of pancreatic β cells.
- **Multiple sclerosis.** A widely used animal model for multiple sclerosis is experimental autoimmune encephalomyelitis (EAE). EAE can be induced relatively easily in Lewis rats by inoculation with homogenized central nervous system myelin or purified myelin antigens together with incomplete Freund's adjuvant. Both acute and chronic relapsing EAE models have been developed.
- **Rheumatoid arthritis.** Immunization of rats with mycobacteria suspended in oil results in the development of adjuvant arthritis, an extensively used animal model of rheumatoid arthritis in humans.

- **Glomerulonephritis.** Injection of BN rats with multiple low doses of HgCl₂ leads to the development of chemically-induced autoimmune glomerulonephritis associated with proteinuria and depositions of autoantibodies to the renal glomerular basement membrane.

Many monoclonal antibodies to rat cell surface antigens and soluble factors have been developed in the last twenty years, and their number is rapidly growing. The availability of these antibodies greatly facilitates studies of the immune system in the rat. Many homologues of human Cluster of Differentiation (CD) antigens have been cloned in the rat allowing detailed analysis of relevant molecules of immunological importance.

Undoubtedly, experimentation in the rat will continue to be a critical tool in biomedical research. Here, we have summarized selected reagents and techniques available from BD Pharmingen for immunological research in the rat animal model.





B Lymphocytes

B and T cells comprise the two major populations of small lymphocytes. B cells mature in the bone marrow and are defined by the expression of the B cell receptor complex (BCR). The BCR consists of membrane bound antibody molecules (sIg or mIg) complexed with the Ig α (CD79a) and Ig β (CD79b) molecules. While sIg recognizes and binds antigen, CD79a and CD79b participate in BCR signal transduction.

B cells generate the humoral immune response and can function as antigen presenting cells for T cell

activation and thus also play a role in cell mediated immune response. One major difference between rat B cells and those in the human or mouse is that in the rat a CD5⁺ subset of B cells has not yet been described. Mature B cells in the rat can easily be distinguished from other leukocytes either on the basis of their expression of sIg or on the basis of their expression of CD45 isoforms unique to B cells. These B cell isoforms of CD45 include the CD45R isoform recognized by HIS24 mAb and CD45RA recognized by OX-33 mAb.

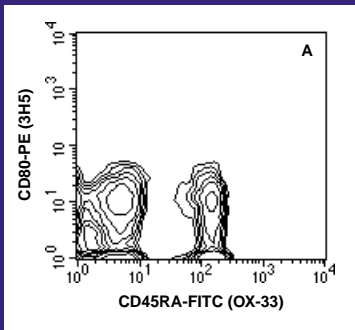


Figure 1

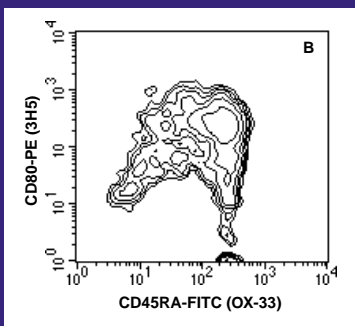


Figure 2

Figure 1,2. Activation-induced changes in the level of expression of the CD80 antigen on fresh splenocytes (A) and cells treated *in vitro* for 72 hours with lipopolysaccharide (LPS) (B). Cells were stained simultaneously with CD45RA-FITC (OX-33) and CD80-PE (3H5).

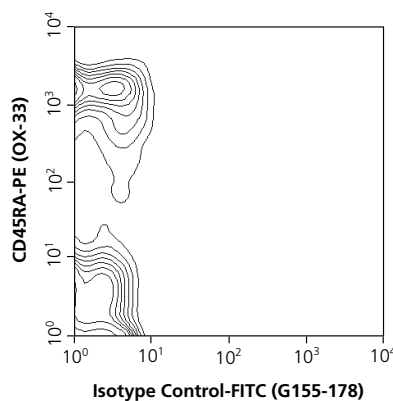


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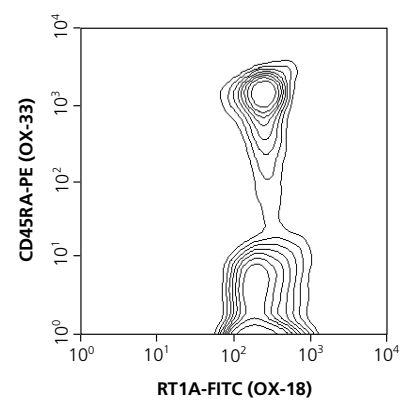


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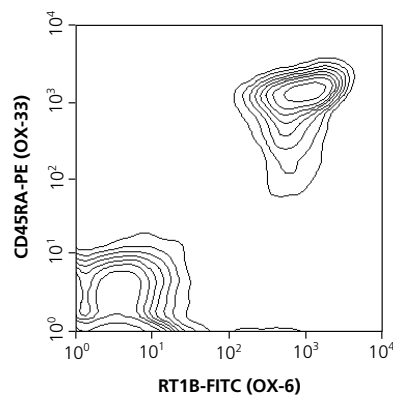


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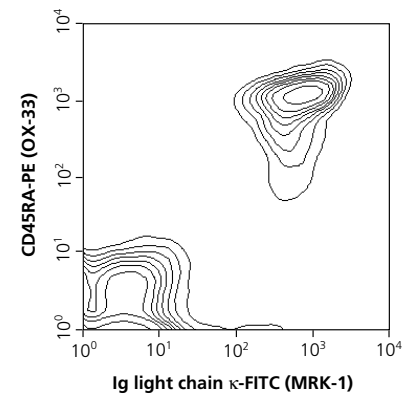
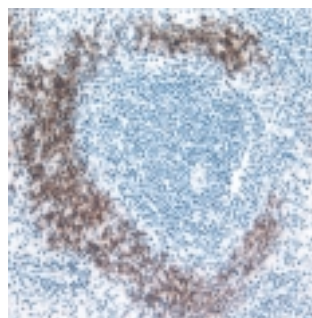
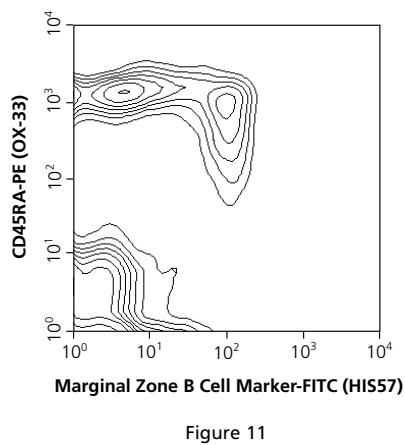
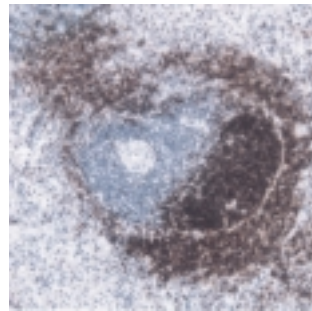
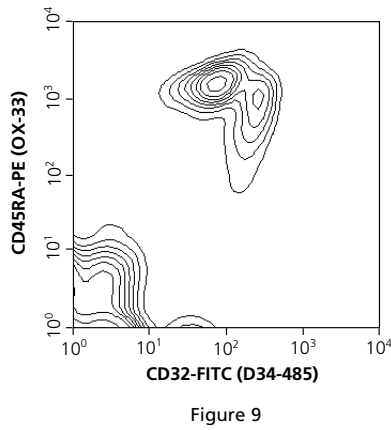
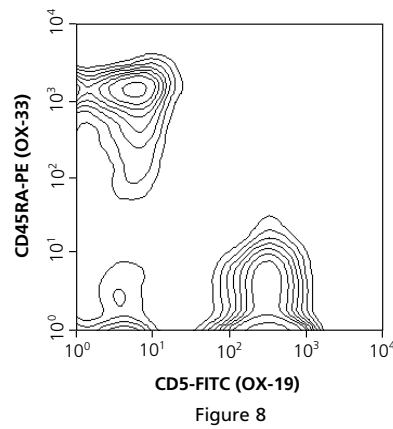
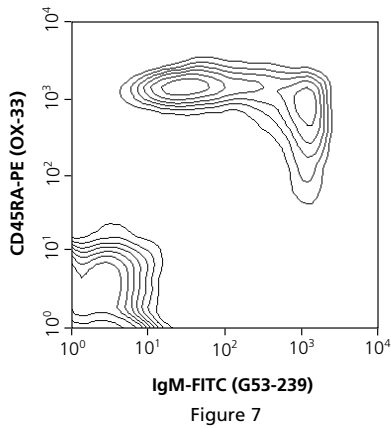
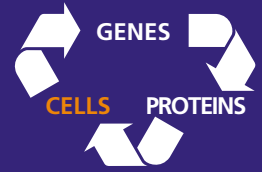
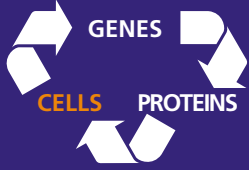


Figure 6



Figures 3-12. Flow cytometry profiles of rat splenocytes using CD45RA-PE (OX-33) antibody to separate B cells from other blood leukocytes. Cells were also stained with a panel of FITC-conjugated antibodies that react with cell surface proteins of interest on B cells (as noted on the x-axis). When available, the same antibody (in purified format) was used for immunohistochemical staining of rat tissue sections.



NEW Rat FcBlock™

B cells also express Fcγ receptors (FcγR) belonging to the immunoglobulin superfamily. The major Fcγ receptors in humans, mice, and rats are FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). These receptors bind to the Fc region of the IgG to help clear immune complexes.

While CD64 binds monomeric IgG, CD32 and CD16 bind to aggregated IgG.

In addition to Mouse FcBlock™, anti-mouse CD32/16, BD PharMingen now offers Rat FcBlock™ (anti-rat CD32) capable of blocking FcγR-mediated binding of aggregated IgG to FcγR bearing cells.

Cells stained after pre-incubation with purified isotype control (no Rat FcBlock™ present)

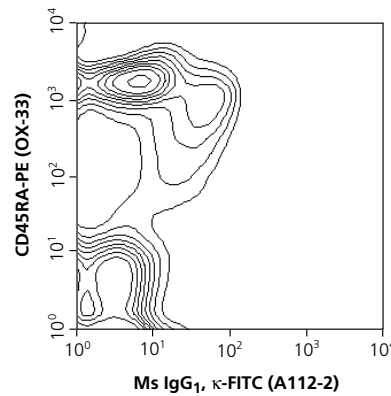


Figure 13

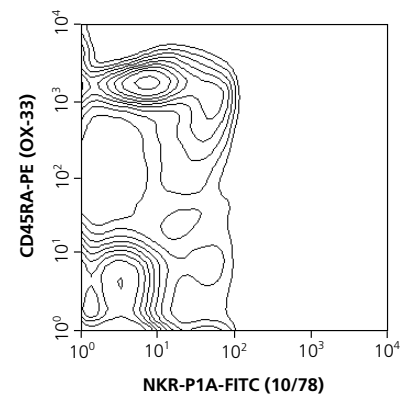


Figure 14

Cells stained after pre-incubation with Rat FcBlock™ (anti-rat CD32, D34-485)

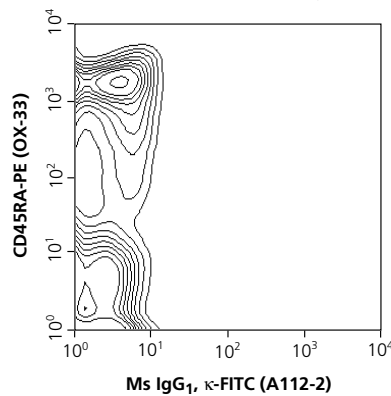


Figure 15

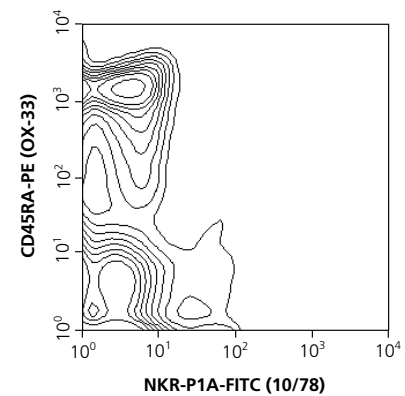


Figure 16

Figures 13-16. Blocking of aggregated Ms IgG1, κ-FITC (A112-2) and anti-NKR-P1A-FITC (10/78) on rat splenocytes by Rat FcBlock™ (CD32, D34-485). A112-2 and 10/78 lots selected for testing contained 2-4% aggregates and approximately 4% of degraded products. FcR-mediated binding of aggregates to B cells (OX-33 positive cells) was eliminated when cells were pre-incubated with Rat FcBlock™ (Figures 15,16) compared to cells pre-incubated with the isotype control (for Rat FcBlock™) (Figures 13,14).

T Lymphocytes

T cells, so named because they mature in the thymus, are a class of small lymphocytes that can be distinguished from other hematopoietic cells based on their function and expression of the T cell receptor (TCR). The TCR complex consists of a dimeric hypervariable T cell receptor, which binds antigen, and an associated invariant CD3 complex, which is involved in TCR signal transduction. CD3-specific monoclonal antibodies (mAb) are well suited as markers for mature T cells and in combination with multi-color immunofluorescence staining and flow cytometric analysis, are

used to study the expression pattern of leukocyte antigens on T cells. Subsets of mature T cells can be delineated by the expression of either CD4 or CD8, and these subsets can be further subdivided according to the types of cytokines the T cells secrete. T cells are important mediators of cytotoxicity and help to coordinate cell mediated and humoral immune responses.

T cells can be classified not only based on the expression of CD4 or CD8 and cytokine secretion profiles but also on the specific alpha or beta chain of TCR they bear on their surfaces.

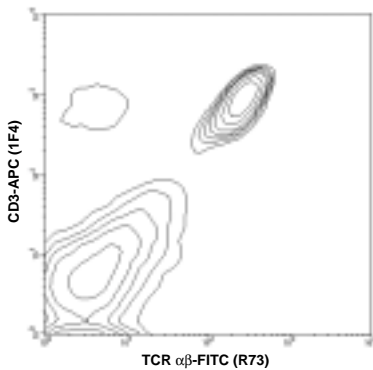
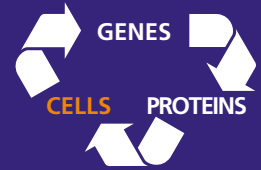


Figure 17

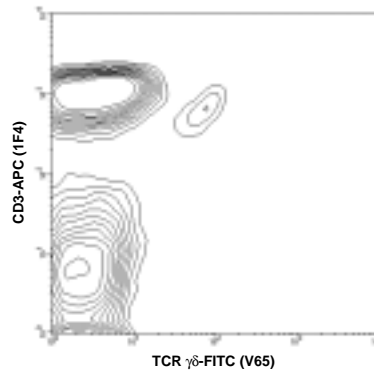


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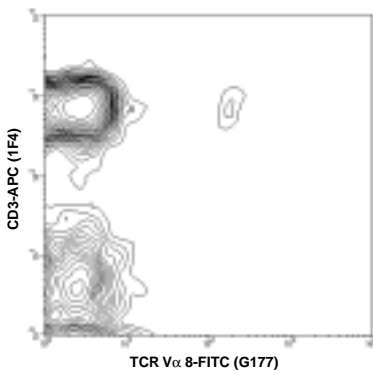


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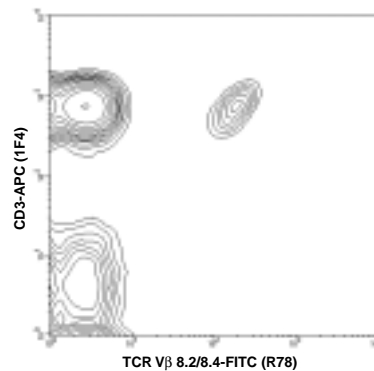
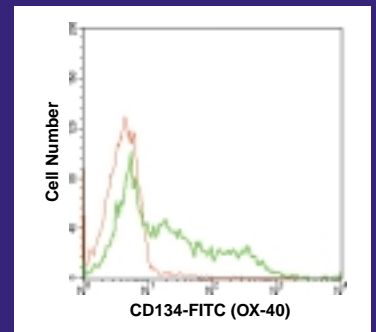
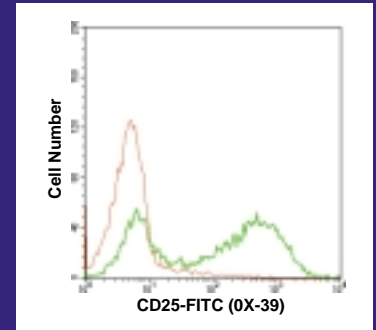


Figure 20

Figures 17-20. Total lymph node cells stained simultaneously with anti-CD3-APC (1F4) and FITC-conjugated (or biotinylated mAb + SAV-FITC) anti-TCR antibodies (as noted on the x-axis).



Figures 21, 22. Expression of CD25 (IL-2R alpha chain) and CD134 (OX-40) on rat spleen T cells (CD3⁺) following *in vitro* incubation for 48 hours with medium alone (red line) or activation for 48 hours with Concanavalin A (green line).

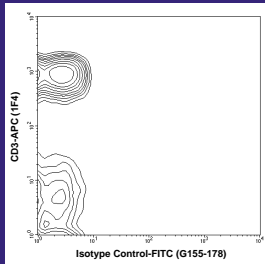
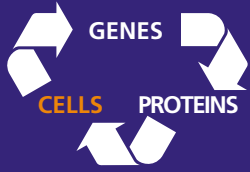
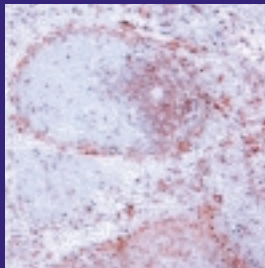


Figure 23



CD2 (OX-34) on frozen spleen

Figure 24

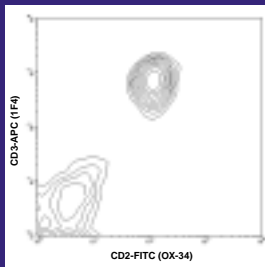
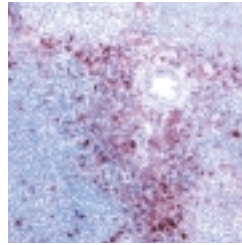


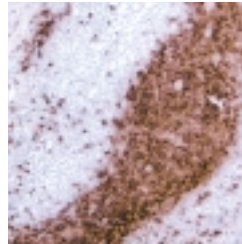
Figure 25

Figures 23-35. Flow cytometric data of rat peripheral blood cells stained with the CD3-APC (1F4) to identify T cells and a panel of FITC-conjugated antibodies (or biotinylated mAb + SAV-FITC) that react with cell-surface proteins of interest on T cells. The same antibody (in purified format) was used for immunohistochemical staining of rat tissue sections.



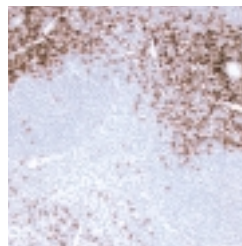
CD4 (OX-38) on frozen spleen

Figure 26



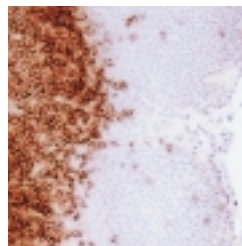
CD5 (OX-19) on frozen spleen

Figure 28



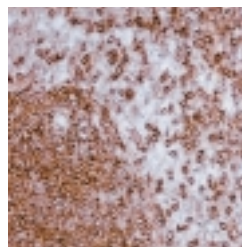
CD8a (OX-8) on paraffin-embedded spleen

Figure 30



Pan-T cell marker (OX-52) on frozen lymph node

Figure 32



CD43 (HIS17) on paraffin-embedded spleen

Figure 34

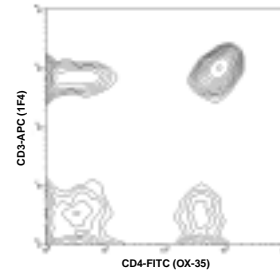


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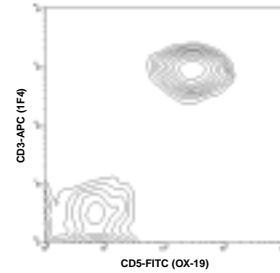


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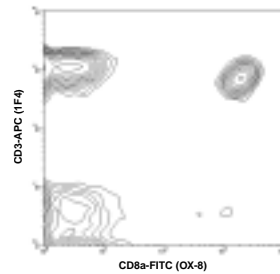


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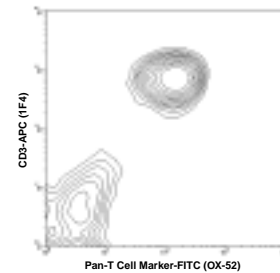


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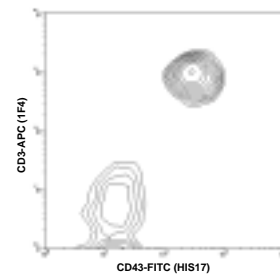


Figure 35

NK Cells

NK cells are large granular lymphocytes that do not express either a BCR or a TCR and are capable of non-MHC restricted cell mediated cytotoxicity without prior sensitization or coating of the target cell with antibody. NK cells are also the effector cells for antibody dependent cell mediated cytotoxicity, a process

in which cells coated with antibody are destroyed following NK cell degranulation and release of perforin and granzymes. NK cells in the rat can be distinguished either by high levels of NKR-P1A expression or by the fact that the majority of NK cells express CD8a, but do not co-express CD3.

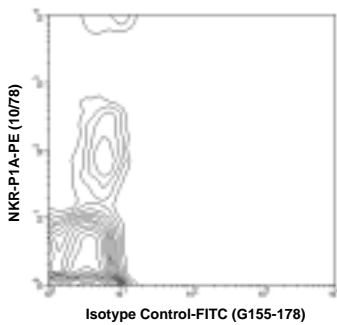
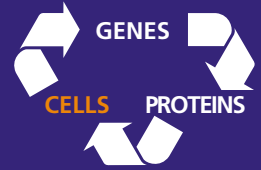


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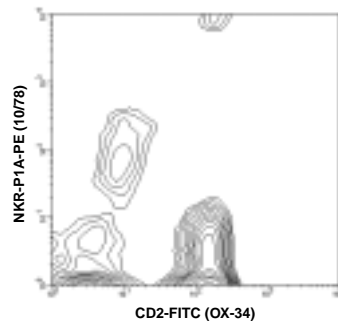


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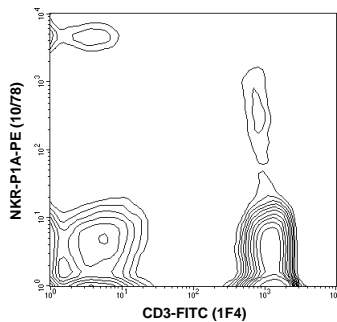


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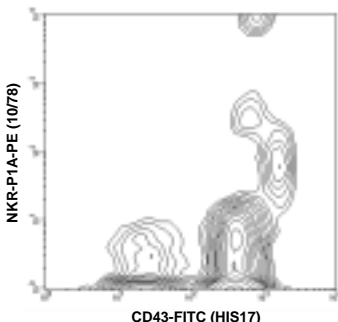


Figure 39

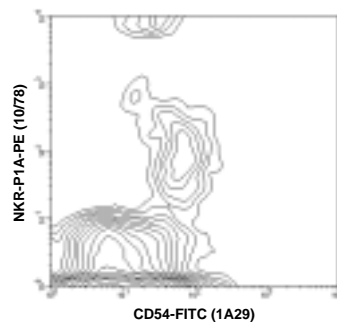
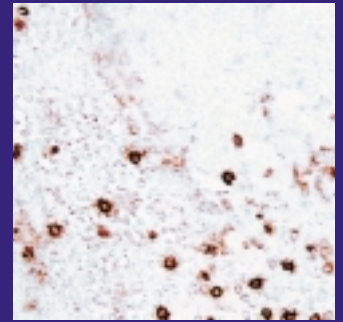


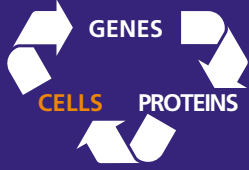
Figure 40

Figures 36-40. Rat peripheral blood cells stained with 1078, a mAb specific for NKR-P1A, and a panel of FITC-conjugated antibodies that react with cell-surface proteins of interest on NK cells.



NKR-P1A (1078), frozen spleen

Figure 41. IHC staining of rat spleen section using the 1078 mAb.

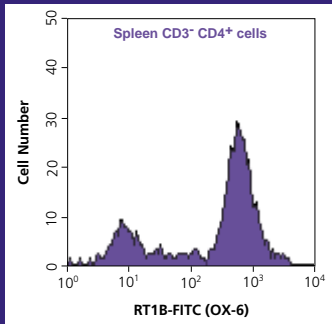
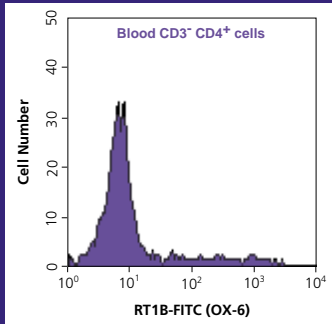


Monocytes/Macrophages

Monocytes and macrophages constitute a second major class of leukocytes. Macrophages play an important role in immune response due to their phagocytic properties, their ability to secrete cytokines and chemokines, and their ability to function as antigen presenting cells. Monocytes differ from macrophages in that monocytes are immature circulating precursors to the various types of tissue associated macrophages. Rat blood monocytes can be distinguished from other leukocytes using an antibody such as HIS48 (specific for monocyte and granulocyte lineages) combined with a gating scheme that takes advantage of the

differential light scattering properties of granulocytes and monocytes/lymphocytes (see next section). A second means for identifying rat monocytes is based on the fact that these cells express CD4 but do not coexpress CD3. This scheme allows effective analysis of the monocytic lineage utilizing BD PharMingen's broad range of antibodies for rat leukocytes.

Following the migration of monocytes to a tissue and their subsequent maturation, phenotypic changes are observed on mature macrophages (Figures 42,43).



Figures 42, 43. Expression of MHC class II RT1B on peripheral blood monocytes (CD3-CD4+) and upregulation on splenic macrophages (CD3-CD4+).

Gated on Monocyte Population

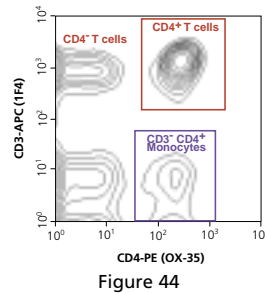


Figure 44

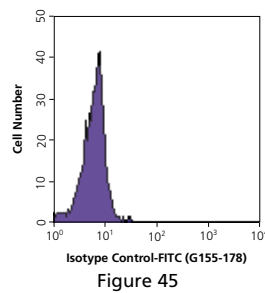


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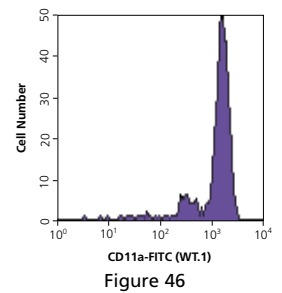


Figure 46

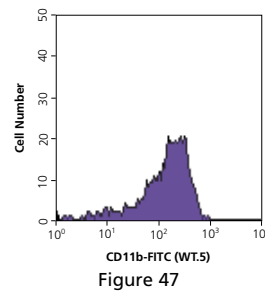


Figure 47

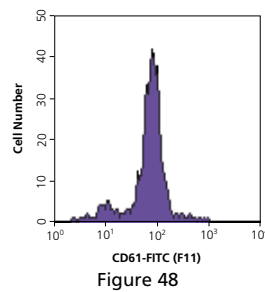


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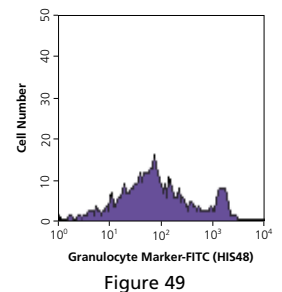


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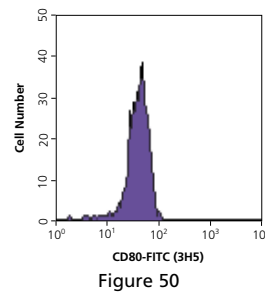


Figure 50

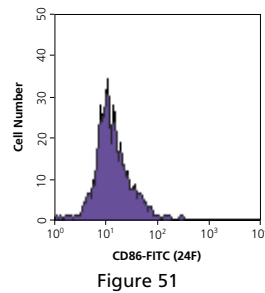
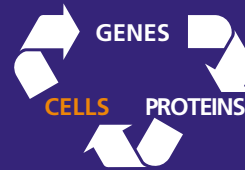


Figure 51

Figures 44-51. Flow cytometric staining of several cell surface antibodies to rat peripheral blood monocytes characterized by the expression of CD4 (OX-35) and lack of CD3 (1F4).

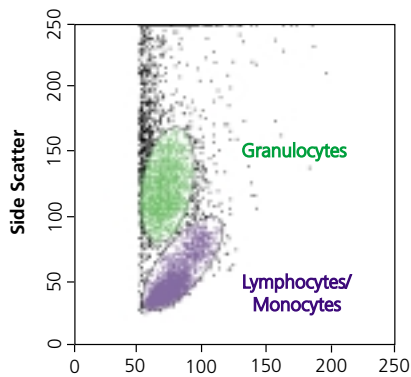


Granulocytes

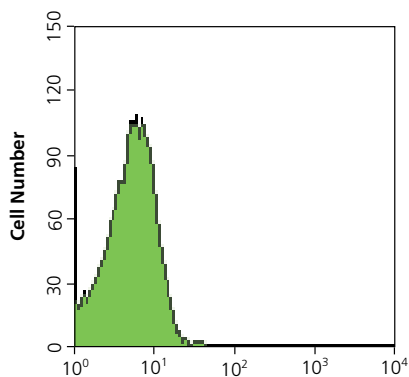
Granulocytes comprise the last major class of hematopoietic cells to be discussed here. Granulocytes characteristically possess numerous cytoplasmic granules. These cells play a role in inflammatory responses

and are capable of phagocytizing opsonized debris. Granulocytes can best be distinguished from other leukocytes when using flow cytometric analysis by their uniquely high levels of side light scatter.

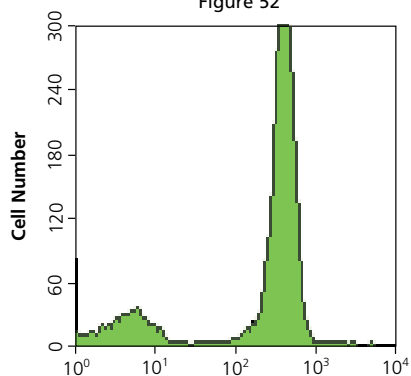
Gated on Granulocyte Population



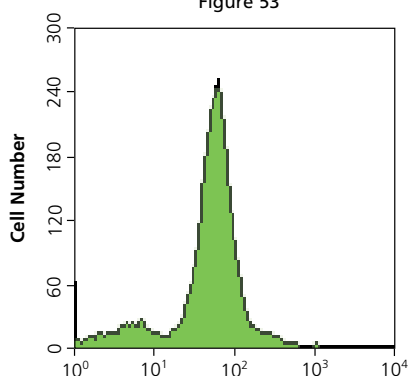
Forward Scatter
Figure 52



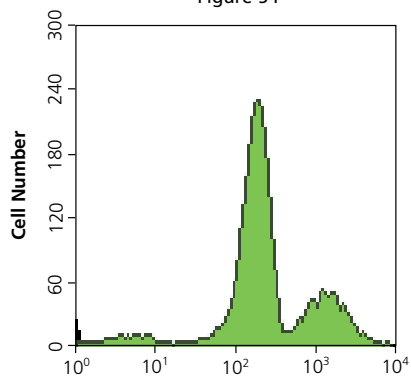
Isotype Control-APC (G155-178)
Figure 53



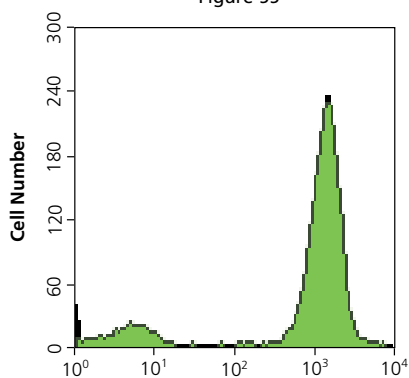
CD11a-APC (WT.1)
Figure 54



CD11b-APC (WT.5)
Figure 55

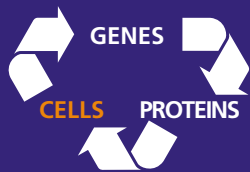


CD61-APC (F11)
Figure 56



Granulocyte Marker-APC (HIS48)
Figure 57

Figures 52-57. Flow cytometric analysis of granulocytes characterized by a high side scatter profile stained with a panel of biotinylated anti-rat cell surface antibodies + SAV-APC.



Cell-Surface Adhesion Molecules

Adhesion molecules play an important role in several aspects of an immune response. These molecules control the homing and migration of leukocytes and mediate many of the direct intercellular interactions essential for successful immune responses. Families of adhesion molecules involved in immune responses include the integrins, the selectins, and members of the Ig superfamily.

Integrins

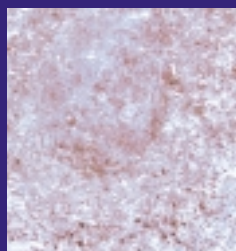
The integrins are a large and diverse family of cell surface molecules that mediate interactions of cells with components of the extracellular matrix, as well as interactions between cells. Integrins are noncovalently linked heterodimers consisting of a large α chain (for example CD11a, b, c and CD49a, b, d, c) paired with a smaller β chain (for example CD18, CD29, and CD61). Much of the diversity within the integrin family is generated by the association of different α and β integrins to form unique integrin heterodimers. These molecules play an important role in inflammatory and immune responses (Figures 58-63).

Selectins

The selectins are a family of carbohydrate binding cell adhesion molecules that play an important role in leukocyte homing. L-selectin (CD62L) is expressed on nearly all circulating leukocytes and is involved in leukocyte homing via interactions with vascular addressins. P-selectin (CD62P) is expressed on activated platelets and endothelial cells where it mediates the adhesion of neutrophils and monocytes to the activated cells. E-selectin (CD62E) is expressed on activated endothelium where it also plays a role in the migration of immune cells (Figure 64).

Ig Superfamily

A third family of adhesion molecules are those which are members of the immunoglobulin (Ig) superfamily. The Ig superfamily includes over 100 members many of which are adhesion molecules (for example CD2, CD31, CD54, and CD106) (Figures 65-66 on page 11).



CD11a (WT.1) on frozen spleen

Figure 58

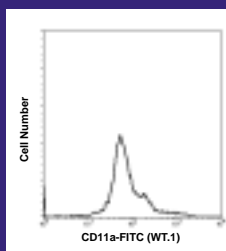
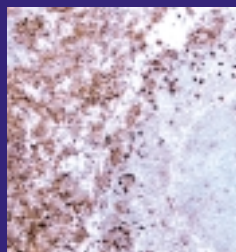


Figure 59



CD18 (WT.3) on paraffin-embedded spleen

Figure 60

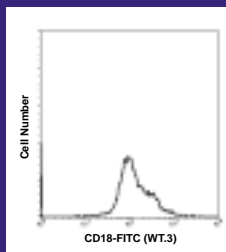
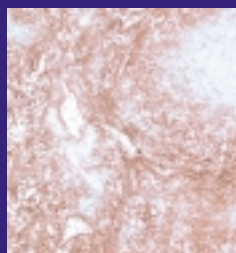


Figure 61



CD29 (Ha2/5) on frozen spleen

Figure 62

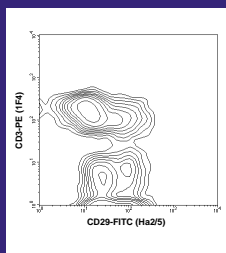


Figure 63

Figures 58-63. Flow cytometric and IHC data generated from rat spleen using antibodies specific for the indicated members of the integrin family.

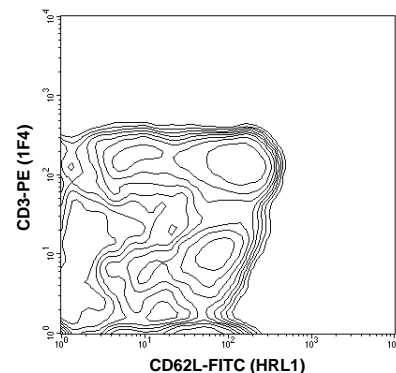
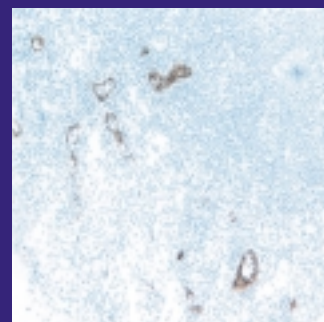
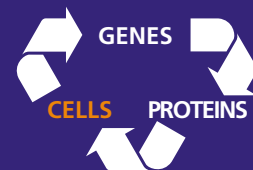


Figure 64. Flow cytometric analysis of rat spleen cells stained simultaneously with CD3-PE (1F4) and CD62L-FITC (HRL1)

Rat MHC Haplotype Chart

RT1 Haplotype	Strains
a	AVN
av1	DA, ACI, ACP, COP
b	BUF, ALB
c	AUG, PVG
d	BVD
dv1	BDIX, TAL
e	BDVII
f	AS2
g	KGH
h	HW
i	BI
j	LEJ
k	WKA/Hok, WKAH, KYN, OKA, SHR
l	LEW
lv1	F344
m	MNR
n	BN, MAXX
o	MR
p	RP
q	NIG III
sa	NSD
sb	WiN
u	WF, TO, AO, LOU, WAG, SDJ, YO, BB/W, DZB



MAdCAM-1 (OST20) on paraffin-embedded spleen

Figure 65

Antibodies to Rat Leukocytes and Related Cells

Specificity	Clone	Isotype	Format	Size	Cat. No.
CD2 (LFA-2)	OX- 34	Mouse IgG _{2a}	Purified	0.5 mg	22001D
			FITC	0.5 mg	22004D
			PE	0.1 mg	22005A
CD3	G4.18	Mouse IgG ₃	NA/LE™	0.5 mg	22010D
			Purified	0.5 mg	22011D
			Biotin	0.5 mg	22012D
			FITC	0.5 mg	22014D
			PE	0.2 mg	22015B
CD3	1F4	Mouse IgM	Purified	0.5 mg	22731D
			FITC	0.5 mg	22734D
			PE	0.1 mg	22735A
			APC	0.1 mg	22739A
CD4	OX-35	Mouse IgG _{2a}	Purified	0.5 mg	22021D
			Biotin	0.5 mg	22022D
			FITC	0.5 mg	22024D
			PE	0.2 mg	22025B
			Cy-Chrome™	0.1 mg	22028A
			APC	0.1 mg	22029A
CD4	OX- 38	Mouse IgG _{2a}	Purified	0.5 mg	22031D
			FITC	0.5 mg	22034D
			PE	0.2 mg	22035B
CD5	OX-19	Mouse IgG ₁	Purified	0.5 mg	22051D
			FITC	0.5 mg	22054D
			PE	0.2 mg	22055B
CD5	HIS47	Mouse IgG _{2a}	Purified	0.5 mg	22061D
CD8a	OX-8	Mouse IgG ₁	Purified	0.5 mg	22071D
			Biotin	0.5 mg	22072D
			FITC	0.5 mg	22074D
			PE	0.2 mg	22075B
CD8a	G28	Mouse IgG _{2a}	Purified	0.1 mg	22421A
			FITC	0.1 mg	22424A
CD8b	341	Mouse IgG ₁	Purified	0.5 mg	22501D
			Biotin	0.5 mg	22502D
			FITC	0.5 mg	22504D

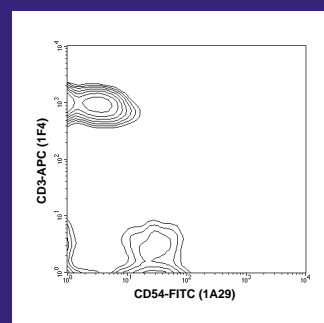
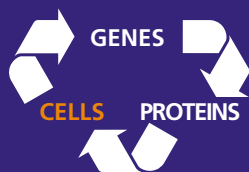


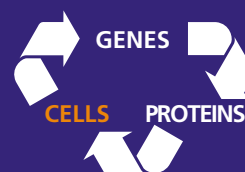
Figure 66

Figures 65, 66. Flow cytometric and IHC data generated from rat spleen using antibodies specific for adhesion molecules belonging to the Ig superfamily.



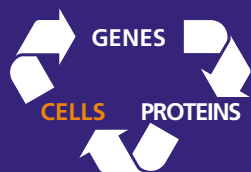
Antibodies to Rat Leukocytes and Related Cells, *continued*

Specificity	Clone	Isotype	Format	Size	Cat. No.
CD9	RPM.7	Mouse IgG ₃	Purified	0.5 mg	22791D
			FITC	0.5 mg	22794D
CD11a (Integrin α L chain LFA-1 α chain)	WT.1	Mouse IgG _{2a}	Purified	0.1 mg	22541A
			FITC	0.5 mg	22544D
			PE	0.2 mg	22545B
CD11b (Integrin α M chain, Mac-1 α chain)	WT.5	Mouse IgA	Purified	0.5 mg	22531D
			Biotin	0.5 mg	22532D
			FITC	0.5 mg	22534D
CD11b/c	OX-42	Mouse IgG _{2a}	NA/LE™	0.5 mg	22080D
			Purified	0.5 mg	22081D
			Biotin	0.5 mg	22082D
			FITC	0.5 mg	22084D
			PE	0.2 mg	22085B
CD15s [Sialyl Lewis X (SLe ^x)]	2H5	Mouse IgM	Purified	0.1 mg	33601A
CD18 (Integrin β 2 chain)	WT.3	Mouse IgG ₁	NA/LE™	0.5 mg	22520D
			Purified	0.5 mg	22521D
			FITC	0.5 mg	22524D
CD25 (IL-2R α chain)	OX - 39	Mouse IgG ₁	Purified	0.1 mg	22091A
			Biotin	0.1 mg	22092A
			FITC	0.5 mg	22094D
			PE	0.2 mg	22095B
New CD26	OX-61	Mouse IgG _{2a}	Purified	0.1 mg	22811A
			FITC	0.1 mg	22814A
			PE	0.1 mg	22815A
New CD27	LG.3A10	Hamster IgG ₁	NA/LE™	0.5 mg	09630D
			Purified	0.1 mg	09631A
			Biotin	0.1 mg	09632A
			PE	0.1 mg	09635A
CD28	JJ319	Mouse IgG ₁	NA/LE™	0.5 mg	22600D
			Purified	0.1 mg	22601A
			Biotin	0.1 mg	22602A
			FITC	0.5 mg	22604D
			PE	0.1 mg	22605A
CD28	JJ316	Mouse IgG ₁	NA/LE™	0.5 mg	22590D
CD29 (Integrin β 1 chain)	Ha2/5	Armenian	NA/LE™	0.5 mg	22630D
		Hamster IgM	Purified	0.5 mg	22631D
			Biotin	0.5 mg	22632D
			FITC	0.5 mg	22634D
CD29 (Integrin β 1 chain)	HM β 1-1	Armenian Hamster IgG, Group 2	Purified	0.5 mg	09871D
CD31 (PECAM-1)	TLD-3A12	Mouse IgG ₁	NA/LE™	0.5 mg	22710D
			Purified	0.5 mg	22711D
			Biotin	0.5 mg	22712D
			PE	0.1 mg	22715A
New CD32	D34-485	Mouse IgG ₁	NA/LE™	0.5 mg	22880D
			Purified	0.5 mg	22881D
			Purified	0.1 mg	22881A
			FITC	0.5 mg	22884D
New OX-40 Ligand	ATM-2	Mouse IgG ₁	Purified	0.5 mg	22821D
			Biotin	0.5 mg	22822D
CD42d	RPM.4	Mouse IgG _{2a}	Purified	0.5 mg	22771D
			FITC	0.5 mg	22774D
CD43 (Leukosialin)	HIS17	Mouse IgG ₁	Purified	0.5 mg	22101D
			Biotin	0.5 mg	22102D
CD44 H (Pgp-1, H-CAM)	OX-49	Mouse IgG _{2a}	Purified	0.5 mg	22111D
			FITC	0.5 mg	22114D
			PE	0.2 mg	22115B
CD45.2 (RT7.2 of Leukocyte Common Antigen)	HIS41	Mouse IgG ₁	Purified	0.1 mg	22121A
			FITC	0.1 mg	22124A
CD45 (Leukocyte Common Antigen)	OX-1	Mouse IgG ₁	Purified	0.5 mg	22131D
			Biotin	0.5 mg	22132D
			FITC	0.5 mg	22134D
			PE	0.2 mg	22135B
			Cy-Chrome™	0.1 mg	22138A



Antibodies to Rat Leukocytes and Related Cells, *continued*

Specificity	Clone	Isotype	Format	Size	Cat. No.
CD45R	HIS24	Mouse IgG _{2b}	Purified	0.5 mg	22161D
			FITC	0.5 mg	22164D
			PE	0.2 mg	22165B
CD45RA	OX-33	Mouse IgG ₁	Purified	0.5 mg	22171D
			Biotin	0.5 mg	22172D
			FITC	0.5 mg	22174D
			PE	0.2 mg	22175B
			Cy-Chrome™	0.1 mg	22178A
CD45RC	OX-22	Mouse IgG ₁	Purified	0.5 mg	22181D
			FITC	0.5 mg	22184D
			PE	0.2 mg	22185B
CD45RC	HIS25	Mouse IgG ₁	Purified	0.5 mg	22221D
CD49a (Integrin α1 chain)	Ha31/8	Armenian	NA/LE™	0.5 mg	22620D
		Hamster IgG, Group 2	Purified	0.5 mg	22621D
CD49b (Integrin α2 chain)	Ha1/29	Armenian	Purified	0.5 mg	22611D
		Hamster IgG, Group 2	FITC	0.5 mg	22614D
CD49d (Integrin α4 chain)	MRα-1	Mouse IgG _{2a}	Purified	0.5 mg	01871D
			FITC	0.1 mg	01874A
CD49e (Integrin α5 chain)	HMα5-1	Armenian Hamster IgG, Group 1	Purified	0.5 mg	01881D
CD53	OX-44	Mouse IgG ₁	Purified	0.5 mg	22751D
			FITC	0.5 mg	22754D
CD54 (ICAM-1)	1A29	Mouse IgG ₁	NA/LE™	0.5 mg	22490D
			Purified	0.5 mg	22491D
			Biotin	0.5 mg	22492D
			FITC	0.5 mg	22494D
			PE	0.2 mg	22495B
CD59	TH9	Mouse IgG ₁	Purified	0.5 mg	22721D
			FITC	0.5 mg	22724D
CD61 (Integrin β3 chain)	F11	Mouse IgG ₁	NA/LE™	0.5 mg	22440D
			Purified	0.5 mg	22441D
			FITC	0.5 mg	22444D
CD61 (Integrin β3 chain)	2C9.G2	Armenian Hamster IgG, Group 1	NA/LE™	0.5 mg	01860D
			Purified	0.5 mg	01861D
			Biotin	0.5 mg	01862D
			FITC	0.5 mg	01864D
			PE	0.2 mg	01865B
CD62L (L-selectin, LECAM-1)	HRL1	Armenian Hamster IgG, Group 2	Purified	0.1 mg	22471A
			Biotin	0.1 mg	22472A
			FITC	0.5 mg	22474D
			PE	0.2 mg	22475B
CD62L (L-selectin, LECAM-1)	HRL2	Armenian Hamster IgG, Group 1	Purified	0.1 mg	22481A
CD62P (P-selectin)	Polyclonal	Rabbit IgG	Purified	0.1 mg	09361A
CD71 (Transferrin Receptor)	OX-26	Mouse IgG _{2a}	Purified	0.5 mg	22191D
			FITC	0.5 mg	22194D
			PE	0.2 mg	22195B
CD80 (B7-1)	3H5	Mouse IgG ₁	NA/LE™	0.5 mg	22660D
			Purified	0.5 mg	22661D
			Biotin	0.5 mg	22662D
			PE	0.2 mg	22665B
CD86 (B7-2)	24F	Mouse IgG ₁	Purified	0.5 mg	22671D
CD90 (Thy-1)	HIS51	Mouse IgG _{2a}	Purified	0.5 mg	22201D
			Biotin	0.5 mg	22202D
			FITC	0.5 mg	22204D
CD90 (Thy-1)	OX-7	Mouse IgG ₁	Purified	0.5 mg	22211D
			Biotin	0.5 mg	22212D
			FITC	0.5 mg	22214D
			PE	0.2 mg	22215B
			PerCP	0.1 mg	2221PA



Antibodies to Rat Leukocytes and Related Cells, *continued*

Specificity	Clone	Isotype	Format	Size	Cat. No.
CD122 (IL-2R β chain)	L316	Mouse IgG ₁	Purified	0.5 mg	22741D
			Biotin	0.5 mg	22742D
			FITC	0.5 mg	22744D
CD134 (OX-40 Antigen)	OX-40	Mouse IgG _{2b}	Purified	0.1 mg	22041A
			Biotin	0.5 mg	22042D
			FITC	0.5 mg	22044D
β 2 Microglobulin	TLD-3H12b	Mouse IgG ₁	Purified	0.5 mg	22801D
			FITC	0.5 mg	22804D
Crry/p65	512	Mouse IgG ₁	Purified	0.5 mg	22581D
Dendritic Cells	OX-62	Mouse IgG ₁	Purified	0.5 mg	22651D
Fas Ligand	MFL4	Armenian	NA/LE™	0.5 mg	22700D
		Hamster	Purified	0.5 mg	22701D
		IgG, Group 3	Biotin	0.5 mg	22702D
Granulocytes	HIS48	Mouse IgM	Purified	0.5 mg	22261D
			Biotin	0.5 mg	22262D
			FITC	0.5 mg	22264D
New Granulocytes	RP-1	Mouse IgG _{2a}	Purified	0.5 mg	22851D
			Biotin	0.5 mg	22852D
			PE	0.1 mg	22855A
New Granulocytes	RP-3	Mouse IgM	NA/LE™	0.5 mg	22840D
			Purified	0.5 mg	22841D
Macrophage Activator	anti-RMA	Mouse IgG ₁	Purified	0.5 mg	22691D
Macrophage Subset	HIS36	Mouse IgG _{2a}	Purified	0.5 mg	22231D
			PE	0.2 mg	22235B
New MAdCAM-1	OST2	Mouse IgG ₁	NA/LE™	0.5 mg	22860D
			Purified	0.1 mg	22861A
New Marginal Zone B Cells	HIS57	Mouse IgG ₁	Purified	0.1 mg	22831A
			Biotin	0.1 mg	22832A
			FITC	0.5 mg	22834D
Mononuclear Phagocyte	1C7	Mouse IgG ₁	Purified	0.5 mg	22451D
NKR-P1A (CD161)	10/78	Mouse IgG ₁	Biotin	0.1 mg	22452A
			Purified	0.5 mg	22641D
			Biotin	0.5 mg	22642D
			FITC	0.5 mg	22644D
			PE	0.2 mg	22645B
Pan-T Cells	OX-52	Mouse IgG _{2a}	Purified	0.5 mg	22251D
			FITC	0.5 mg	22254D
QCA-1	HIS45	Mouse IgG ₁	Purified	0.5 mg	22241D
New VCAM-1 (CD106)	MR106	Mouse IgG ₁	Purified	0.5 mg	22681D
			PE	0.1 mg	22685A

Antibodies to Rat Major Histocompatibility Complex (MHC) Antigens

Specificity	Clone	Isotype	Format	Size	Cat. No.
RT1A	OX-18	Mouse IgG ₁	Purified	0.5 mg	22301D
			Biotin	0.5 mg	22302D
			FITC	0.5 mg	22304D
			PE	0.1 mg	22305A
RT1A a, b	C3	Rat IgG _{2b}	Purified	0.5 mg	22351D
			Biotin	0.1 mg	22352A
			FITC	0.5 mg	22354D
RT1B a, b, I	B5	Rat IgM	Purified	0.1 mg	22371A
			FITC	0.1 mg	22374A
RT1B	OX-6	Mouse IgG ₁	NA/LE™	0.5 mg	22380D
			Purified	0.5 mg	22331D
			Biotin	0.5 mg	22332D
			FITC	0.5 mg	22334D
			PE	0.2 mg	22335B
			PerCP	0.1 mg	2233PA
RT1B (non-n haplotypes)	HIS19	Mouse IgG ₁	Purified	0.5 mg	22311D
RT1D	OX-17	Mouse IgG ₁	Purified	0.5 mg	22341D
			FITC	0.5 mg	22344D

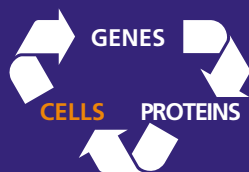


Antibodies to Rat T-Cell Receptors (TCR)

Specificity	Clone	Isotype	Format	Size	Cat. No.
$\alpha\beta$ TCR	R73	Mouse IgG ₁	NA/LE™	0.5 mg	22280D
			Purified	0.5 mg	22281D
			Biotin	0.5 mg	22282D
			FITC	0.5 mg	22284D
			PE	0.2 mg	22285B
			PerCP	0.1 mg	2228PA
$\gamma\delta$ TCR	V65	Mouse IgG ₁	Purified	0.5 mg	22461D
			Biotin	0.1 mg	22462A
			FITC	0.5 mg	22464D
			PE	0.2 mg	22465B
$\gamma\delta$ TCR Subset	V45	Mouse IgG ₁	Purified	0.5 mg	22511D
			Biotin	0.5 mg	22512D
V α 4 TCR	G99	Mouse IgG ₁	Purified	0.5 mg	22561D
V α 8 TCR	G177	Mouse IgG ₁	Purified	0.5 mg	22551D
V β 8.21 and V β 8.4a TCR	R78	Mouse IgG ₁	Purified	0.5 mg	22391D
			FITC	0.1 mg	22394A
V β 8.5 TCR	B73	Mouse IgG ₁	Purified	0.5 mg	22401D
V β 10 TCR	G101	Mouse IgG2 _a	FITC	0.5 mg	22414D
V β 16 TCR	HIS42	Mouse IgG2 _b	Purified	0.5 mg	22291D

Isotype Controls

Specificity	Clone	Format	Size	Cat. No.
Mouse IgG ₁ , κ	MOPC-31C	Purified	0.5 mg	03251D
Mouse IgG ₁ , κ (anti-KLH)	A112-2	NA/LE™	0.5 mg	03210D
		Purified	0.5 mg	03211D
		Biotin	0.25 mg	03212C
		FITC	0.25 mg	03214C
		PE	0.1 mg	03215A
		Cy-Chrome™	0.1 mg	03218A
PerCP	0.1 mg	0321PA		
Mouse IgG ₁ , κ (b allotype, anti-KLH)	A115-2	Purified	0.5 mg	03221D
Mouse IgG ₁ , λ (anti-TNP)	A111-3	Purified	0.5 mg	03191D
Mouse IgG ₁ , λ	S1-68.1	Purified	0.5 mg	03011D
Mouse IgG2 _a , κ (anti-TNP)	G155-178	NA/LE™	0.5 mg	03020D
		Purified	0.5 mg	03021D
		Biotin	0.25 mg	03022C
		FITC	0.25 mg	03024C
		PE	0.1 mg	03025A
Cy-Chrome™	0.1 mg	03028A		
Mouse IgG2 _a , λ	HOPC-1	Purified	0.5 mg	03031D
Mouse IgG2 _b , κ (anti-TNP)	49.2	Biotin	0.25 mg	03042C
		FITC	0.25 mg	03044C
		PE	0.1 mg	03045A
Mouse IgG2 _b , λ	G11-59	Purified	0.5 mg	03051D
Mouse IgG ₃ , κ (anti-Fructosan)	J606	NA/LE™	0.5 mg	03060D
Mouse IgG ₃ , κ (anti-KLH)	A112-3	Purified	0.5 mg	03201D
		Biotin	0.25 mg	03202C
		FITC	0.25 mg	03204C
		PE	0.1 mg	03205A
Mouse IgG ₃ , λ	G19-143	Purified	0.5 mg	03071D
Mouse IgM, κ (anti-TNP)	G155-228	Purified	0.5 mg	03081D
		Biotin	0.25 mg	03082C
		FITC	0.25 mg	03084C
Mouse IgM, λ (anti-Dextran)	MOPC-104E	Purified	0.5 mg	03091D
Mouse IgA, κ	M18-254	Purified	0.5 mg	03101D
		Biotin	0.25 mg	03102C
		FITC	0.25 mg	03104C



Isotype Controls, *continued*

Specificity	Clone	Format	Size	Cat. No.
Mouse IgA, λ	MOPC-315	Purified	0.5 mg	03111D
Mouse IgE, κ (anti-dansyl)	27-74	Purified	0.5 mg	03131D
Mouse IgE, κ (anti-TNP)	C38-2	Purified	0.5 mg	03231D
Mouse IgE, κ (b allotype, anti-TNP)	C48-2	Purified	0.5 mg	03241D
Hamster IgG, κ group 1, (anti-TNP)	A19-3	NA/LE™	0.5 mg	11150D
		Purified	0.5 mg	11151D
		Biotin	0.25 mg	11152C
		FITC	0.25 mg	11154C
		PE	0.1 mg	11155A
		Cy-Chrome™	0.1 mg	11158A
		APC	0.1 mg	11159A
Hamster IgG, λ group 1, (anti-TNP)	G235-2356	NA/LE™	0.5 mg	11120D
		Purified	0.5 mg	11121D
		Biotin	0.25 mg	11122C
		FITC	0.25 mg	11124C
		PE	0.1 mg	11125A
		Cy-Chrome™	0.1 mg	11128A
		Hamster IgG, λ group 2, (anti-KLH)	Ha4/8	NA/LE™
Purified	0.5 mg			11141D
Biotin	0.25 mg			11142C
FITC	0.25 mg			11144C
PE	0.1 mg			11145A
Cy-Chrome™	0.1 mg			11148A
APC	0.1 mg			11149A
Hamster IgG, λ group 3, (anti-TNP)	A19-4	NA/LE™	0.5 mg	11160D
		Purified	0.5 mg	11161D
		Biotin	0.25 mg	11162C
		FITC	0.25 mg	11164C
		PE	0.1 mg	11165A
Hamster IgM, λ (anti-TNP)	G235-1	NA/LE™	0.5 mg	11130D
		Purified	0.5 mg	11131D
		Biotin	0.25 mg	11132C
		FITC	0.25 mg	11134C

Cytokines

The development and function of the immune system depends on the production and response to cytokines. Cytokines are pleiotropic mediators capable of stimulating multiple biological effects.

Cytokines have taken center stage in many clinical and basic research studies because they play such a primary role in transmitting regulatory signals between various types of cells. The intercellular communication system consisting of cytokines and their receptors is comprised of multiple stimulatory and inhibitory cytokines. The complexity of the cytokine network often necessitates a multi-parameter approach to study the functional roles played by a particular cytokine, since each cytokine's role may be influenced by other cytokines which are present in

the experimental system. BD PharMingen has designed its line of cytokine reagents in response to the needs of researchers for simultaneous analysis of multiple cytokines.

BD PharMingen offers a growing line of reagents for the study of rat cytokines and chemokines including recombinant rat cytokines, specific antibodies to rat cytokines and chemokines, and optimized immunoassays. These antibodies are offered in formats useful for detection of cytokine producing cells by flow cytometry, determination of cytokine protein levels by sandwich ELISA, as well as functional analysis utilizing biologically active recombinant proteins, and neutralizing anti-cytokine antibodies.

Detection of Intracellular Cytokines by Flow Cytometry

Multicolor immunofluorescent staining with antibodies against intracellular cytokines and cell surface markers provides a high-resolution method to determine the nature and frequency of cells which express a particular cytokine(s). For example, staining of an individual cell surface antigen and two cytoplasmic cytokines has been used to identify and enumerate cell types that express cytokines in either a polarized (*e.g.*, Th1- versus Th2-like cells) or unpolarized (*e.g.*, Th0-like cells) pattern. In addition to enabling highly specific and sensitive measurements of several parameters for individual cells simultaneously, this method has the capacity for rapid analysis of large numbers of cells, which are

required for making statistically significant measurements.

In addition to fluorochrome-conjugated antibodies for intracellular staining of rat cytokines, BD PharMingen provides Cytofix/Cytoperm Intracellular flow cytometry kits that provide fixation and permeabilization buffers and protein transport inhibitors. Also now available are RiCK (Rat intracellular CytoKine) positive control cells. These cell suspensions contain fixed, non-permeabilized rat lymphoid cells that express easily detected levels of intracellular cytokines and can thus be used as positive controls for experiments.

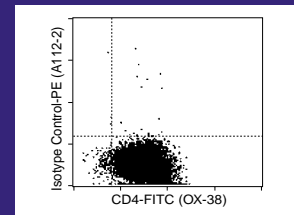
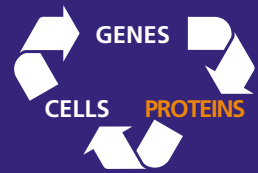


Figure 67

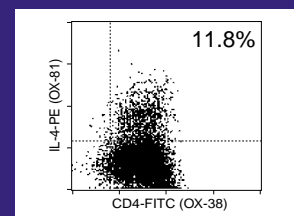


Figure 68

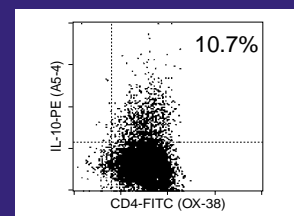


Figure 69

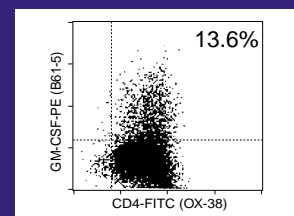


Figure 70

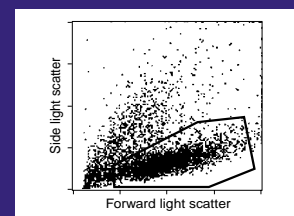
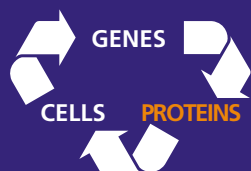


Figure 71

Figures 67-71. RiCK-2 positive control cells stained with FITC-conjugated mouse anti-rat CD4 (OX-38), washed, permeabilized, and subsequently stained with PE mouse IgG1 isotype control (A112-2), PE-mouse anti-rat IL-4 antibody (OX-81), PE-mouse anti-rat IL-10 (A5-4) or PE mouse anti-rat GM-CSF (B61-5). Figure 74. The light-scattering characteristics for RiCK-2 cells are shown.



Antibodies for Intracellular Cytokine and Chemokine Flow Cytometry

Antibodies for Rat Cytokines

Specificity	Clone	Isotype	Format	Application	Size	Cat. No.
IL-4	OX-81	Mouse IgG ₁	Purified	IC/Flow blocking	0.1 mg	24051A
			PE	IC/Flow staining	0.1 mg	24054A
IFN- γ	DB-1	Mouse IgG ₁	Purified	IC/Flow blocking	50 ng	20441N
			FITC	IC/Flow staining	100 tests	20444X
			PE	IC/Flow staining	100 tests	20445X
IL-10	A5-4	Mouse IgG _{2b}	Purified	IC/Flow blocking	0.1 mg	24091A
			PE	IC/Flow staining	0.1 mg	24095A
TNF- α	TN3-19.12	Hamster IgG	Purified	IC/Flow blocking	0.1 mg	23351A
			PE	IC/Flow staining	0.1 mg	23355A
GM-CSF	B61-5	Mouse IgG ₁	Purified	IC/Flow blocking	0.1 mg	24131A
			PE	IC/Flow staining	0.1 mg	24135A

Antibodies for Rat Chemokines

MCP-1	2H5	Hamster IgG	Purified	IC/Flow blocking	0.1 mg	18241A
			PE	IC/Flow staining	0.1 mg	18245A

Cell Fixation and Permeabilization Kits and Components

Cytofix/Cytoperm™ Kit	250 tests	2075KK
Cytofix/Cytoperm™ Kit with GolgiStop™	250 tests	2076KK
Cytofix/Cytoperm™ Kit with GolgiPlug™	250 tests	2300KK
GolgiStop™ (with Monensin)	1.0 ml	2092KZ
GolgiPlug™ (with Brefeldin A)	0.7 ml	2301KZ
Cytofix/Cytoperm™ Buffer	100 mls	2090KZ
Perm/Wash™ Buffer	100 mls	2091KZ
NEW BrdU Flow Kit	50 tests	2354KK

Rat Intracellular Cytokine Positive Control Cells

RiCK-2 (Positive for IL-4, IL-10, GM-CSF, IFN- γ , TNF- α)	5x10 ⁶ cells	24142Z
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Antibodies for Rat Cytokine Receptors

IL-2R α	OX-39	Mouse IgG1	Purified	Flow Cytometry	0.5 mg	22091D
			Biotin		0.5 mg	22092D
			FITC		0.5 mg	22094D
			PE		0.2 mg	22095B
IL-2R β	L316	Mouse IgG1	Purified	Flow Cytometry	0.5 mg	22741D
			Biotin		0.5 mg	22742D
			FITC		0.5 mg	22744D

Reagents for Immunofluorescent Staining of Cell Suspensions

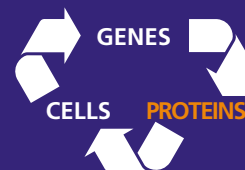
PharMingenStain Buffer (with FBS)	20151E
PharMingenStain Buffer (with BSA)	20161E
Cytofix™ Buffer	2014KZ

Neutralizing Rat Cytokine Antibodies

IL-2	Polyclonal	Rabbit Ig	NA/LE™	Neutralization	0.5 mg	24030D
IL-4	OX-81	Mouse IgG ₁	NA/LE™	Neutralization	0.5 mg	24050D
TNF- α	TN3-19.12	Hamster Ig	NA/LE™	Neutralization	0.5 mg	23350C

Recombinant Rat Cytokines and Chemokines

IL-2	Bioactive/ELISA	5 μ g	25001V
IL-4	Bioactive/ELISA	5 μ g	25011V
IL-6	Bioactive/ELISA	5 μ g	25081V
IL-10 (ELISA Standard)	ELISA only	5 μ g	25071V
GM-CSF	Bioactive/ELISA	5 μ g	25051V
IFN- γ	Bioactive	2 μ g	25061V
TNF- α	Bioactive/ELISA	5 μ g	25031V
MCP-1	Bioactive/ELISA	5 μ g	25041V



BD OptEIA™ Rat Cytokine ELISA Sets

BD OptEIA™ ELISA Sets are specially formulated to facilitate accurate measurement of soluble proteins in tissue culture supernatants, sera, plasma, and other complex biological fluids. Each set consists of pre-titered capture and detection antibodies along with lyophilized recombinant cytokine standard and horseradish peroxidase conjugate sufficient for 20

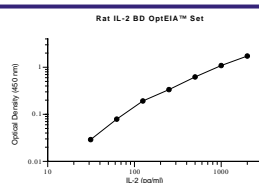
96-well plates. The BD OptEIA™ Set reagents are specifically formulated to reduce background and non-specific binding caused by autoantibodies, rheumatoid factor, and plasma proteins such as complement, fibronectin and Fc receptors. Typical standard curves from BD OptEIA™ Sets for rat IL-2, IL-4, IL-10, IFN- γ , TNF- α , and MCP-1 are shown below.

US Price 550

Rat IL-2 OptEIA™ Set; Cat. No. 2628KI

Contents:

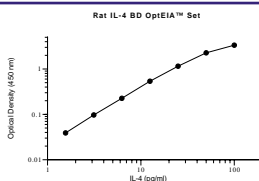
- Capture Antibody: Anti-rat IL-2
- Detection Antibody: Biotinylated anti-rat IL-2
- Enzyme Reagent: HRP-conjugated avidin
- Standard: Recombinant rat IL-2 (lyophilized)
- Standard Curve Range: 31.3 - 2000 pg/ml



Rat IL-4 OptEIA™ Set; Cat. No. 2631KI

Contents:

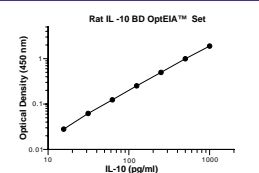
- Capture Antibody: Anti-rat IL-4
- Detection Antibody: Biotinylated anti-rat IL-4
- Enzyme reagent: HRP-conjugated avidin
- Standard: Recombinant rat IL-4 (lyophilized)
- Standard Curve Range: 1.6 - 100 pg/ml



Rat IL-10 OptEIA™ Set; Cat. No. 2611KI

Contents:

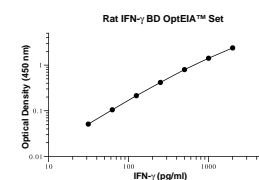
- Capture Antibody: Anti-rat IL-10
- Detection Antibody: Biotinylated anti-rat IL-10
- Enzyme Reagent: HRP-conjugated avidin
- Standard: Recombinant rat IL-10 (lyophilized)
- Standard Curve Range: 15.6 - 1000 pg/ml



Rat IFN- γ OptEIA™ Set; Cat. No. 2696KI

Contents:

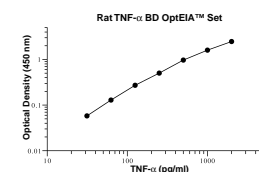
- Capture Antibody: Anti-rat IFN- γ
- Detection Antibody: Biotinylated anti-rat IFN- γ
- Enzyme Reagent: HRP-conjugated avidin
- Standard: Recombinant rat IFN- γ (lyophilized)
- Standard Curve Range: 31.3 - 2000 pg/ml



Rat TNF- α OptEIA™ Set; Cat. No. 2697KI

Contents:

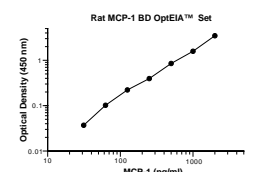
- Capture Antibody: Anti-rat TNF- α
- Detection Antibody: Biotinylated anti-rat TNF- α
- Enzyme Reagent: HRP-conjugated avidin
- Standard: Recombinant rat TNF- α (lyophilized)
- Standard Curve Range: 31.3 - 2000 pg/ml

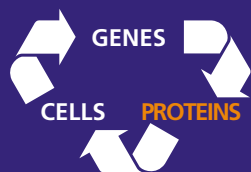


Rat MCP-1 OptEIA™ Set; Cat. No. 2610KI

Contents:

- Capture Antibody: Anti-rat MCP-1
- Detection Antibody: Biotinylated anti-rat MCP-1
- Enzyme Reagent: HRP-conjugated avidin
- Standard: Recombinant rat MCP-1 (lyophilized)
- Standard Curve Range: 31.3 - 2000 pg/ml





OptEIA™ Sets General ELISA Reagents

TMB Substrate Reagent Set;
Cat. No. 2642KK

Assay Diluent;
Cat. No. 26411E

Contents:

- Substrate Reagent A, 300 ml
- Substrate Reagent B, 300 ml

Volume: 500 ml

Assay Diluent contains animal serum in a buffered solution with 0.15% ProClin-150 as preservative. Intended uses are to block ELISA plates, to dilute the recombinant Standards and samples, and to prepare Working Detector for the BD OptEIA™ Sets ELISA Reagents.

Substrate Reagent A contains hydrogen peroxide in a buffered solution. Substrate Reagent B contains 3, 3', 5, 5'-tetramethylbenzidine in organic solvent. When mixed together, TMB substrate solution reacts with peroxidase-labeled conjugates to develop a blue color. After addition of stopping solution (1M phosphoric or 2N sulfuric acid), the color changes from blue to yellow and the microwells are read at 450 nm.

Matched Antibody ELISA Pairs and Protein Standards

BD Pharmingen's extensive portfolio of matched antibody ELISA pairs and protein standards for rat cytokines and chemokines offer the researcher:

- Flexible assay development
- Lower cost of ELISA analysis per sample
- Reagent use for other applications such as western blotting

Matched Antibody Pairs and Protein Standards for Rat Cytokine and Chemokine ELISA

Specificity	Description	Clone	Isotype	Format	Size	Cat. No.
IL-2	Capture	Polyclonal	Rabbit Ig	Purified	0.5 mg	24031D
	Detection	A38-3	Mouse IgG ₁	Biotin	0.5 mg	24042D
	Standard	Rec. IL-2		Recombinant	5 µg	25001V
IL-4	Capture	OX-81	Mouse IgG ₁	Purified	0.5 mg	24051D
	Detection	Polyclonal	Rabbit Ig	Biotin	0.5 mg	24112D
	Standard	Rec. IL-4		Recombinant	0.5 µg	25011V
IL-4	Capture	OX-81	Mouse IgG ₁	Purified	0.5 mg	24051D
	Detection	B11-3	Mouse IgG ₁	Biotin	0.5 mg	24112D
	Standard	Rec. IL-4		Recombinant	0.5 µg	25011V
IL-10	Capture	A5-7	Mouse IgG ₁	Purified	0.5 mg	24061D
	Detection	A5-5	Mouse IgG ₁	Biotin	0.5 mg	24072D
	Standard	Rec. IL-10		Recombinant	0.5 µg	25071V
GM-CSF	Capture	B61-5/B61-9	Mouse IgG ₁	Purified	0.5 mg	24181D
	Detection	B61-10	Mouse IgG ₁	Biotin	0.5 mg	24172D
	Standard	Rec. GM-CSF		Recombinant	0.5 µg	25051V
MCP-1	Capture	C4	Mouse IgG ₁	Purified	0.5 mg	24011D
	Detection	B4	Mouse IgG ₁	Biotin	0.5 mg	24022D
	Standard	Rec.MCP-1		Recombinant	5 µg	24041V
TNF-α	Capture	TN3-19.12	Hamster Ig	Purified	0.5 mg	23351D
	Detection	Polyclonal	Rabbit Ig	Biotin	0.5 mg	23442D
	Standard	Rec.TNF-α		Recombinant	5 µg	25031V

Cell Biology: Apoptosis and Neurobiology

Apoptosis

Apoptosis, a form of programmed cell death, is a normal physiological process which occurs during normal embryonic development and in the maintenance of tissue homeostasis. Inappropriate induction of apoptosis has broad ranging pathologic implications and as a result, the apoptosis field is of increasing scientific interest, with thousands of new publications occurring every year. BD PharMingen offers a number of reagents designed to identify, quantitate, and characterize apoptosis.

Neurobiology

In addition to our apoptosis line of products, we have a number of antibodies for the study of cellular and neurobiological systems. BD PharMingen offers a wide variety of antibodies and other reagents for neurobiological research in the rat, including antibodies to neurotransmitters and their receptors, glutamate receptors and structural proteins, tumor suppressors, oncogenes, cell cycle-related proteins, and a wide variety of other intracellular proteins.

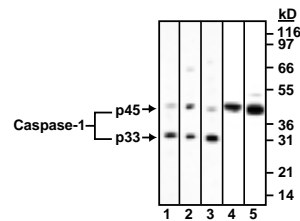


Figure 72. Western blot analysis of caspase-1. HeLa human cervical carcinoma (lane 1), 293 adenovirus-transformed human embryonic kidney (lane 2), Jurkat T cell (lane 3), BALB/c thymocytes (lane 4) and DC-3 SV40-transformed, rat ovarian granulosa cells (lane 5) probed with anti-caspase-1 (clone B24-2, Cat. No. 66441A). Caspase-1 is identified as 45 kD (precursor) and 33 kD (intermediate) bands. In rat species, clone B24-2 primarily detects the 45 kD form of the protein.

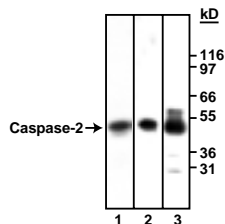


Figure 73. Western blot analysis of caspase-2. Jurkat T cell (lane 1), mouse myeloblasts (lane 2) and DC-3 SV40-transformed, rat ovarian granulosa cells (lane 3) probed with anti-caspase-2 (clone G310-1248, Cat. No. 13951A). The G310-1248 antibody identifies caspase-2 as an ~48 kD band.

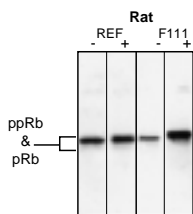


Figure 74. Western blot analysis of Rb, clone G3-245 (Cat. No. 14001A) in cell cycling (+) and quiescent (-) rat cell cultures. Most highly phosphorylated species of Rb are seen in cycling compared to quiescent cultures. REF, primary rat embryo fibroblasts. F111, established rat fibroblasts. ppRb and pRb represent different phosphorylation states of Rb. The higher molecular weight bands (116 kD) are more highly phosphorylated than the lower bands (110 kD).

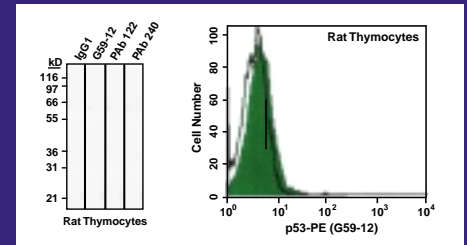
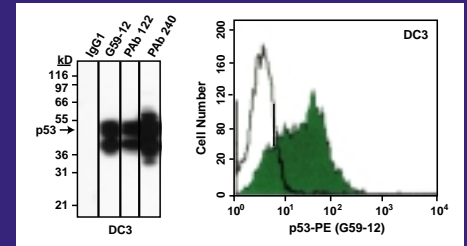
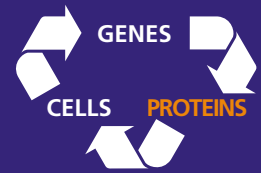


Figure 75. Analysis of p53 expression in SV40-transformed rat ovarian cells (DC3) and rat thymocytes. Cells were analyzed by flow cytometry using a p53-PE antibody (G59-12) (green histogram) or an IgG1-PE isotype control (MOPC-21) (open histogram), or by western blot analysis using a panel of p53 antibodies [G59-12 (Cat. no. 14211A), PAb 122 (Cat. no. 14091A), or PAb 240 (Cat. No. 14461A)] or an IgG1 isotype control (MOPC-21, Cat. No. 03171D). The results show that p53 was detected in DC3 cells but not in rat thymocytes. The PE-conjugated p53 and IgG1 antibodies are sold as a set (Cat. No. 6733KK).

Cell Biology: Apoptosis and Neurobiology, *continued*

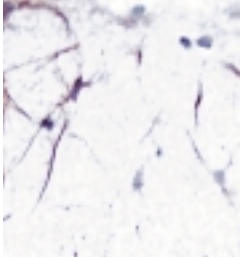


Figure 76. Acetone-fixed, frozen tissue section of rat brain stained for somatostatin (clone YC7, Cat. No. 60132A).

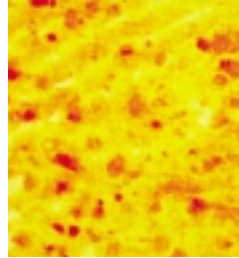


Figure 77. Paraformaldehyde-perfused, frozen rat brain stained with anti-TrkA (Cat. No. 68266E).

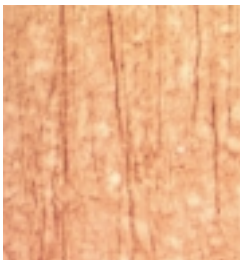


Figure 78. Frozen rat brain tissue stained for 5-HT2AR (clone G186-1117, Cat. No. 60231A).



Figure 79. Western blot analysis of 5-HT2AR in rat brain synaptic membranes. Blots were incubated with either G186-1117. (lane 1), G186-1117, absorbed with recombinant GST-5-HT2AR fusion protein (lane 2), or G186-1117 absorbed with an irrelevant (GST-p16) fusion protein (lane 3). The antibody recognizes 5-HT2AR as an ~55 kD band.

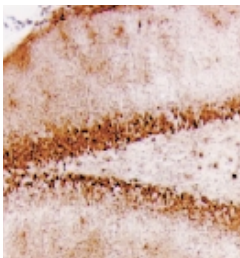


Figure 80. Frozen rat brain tissue (hippocampus) stained for 5-HT2BR (clone A72-1, Cat. No. 60531A).

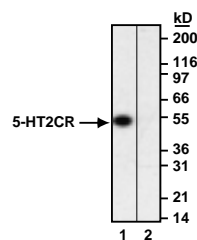


Figure 81. Western blot analysis of 5-HT2CR in rat brain synaptic plasma membrane. (Lane 1), clone A4-2 (Cat. no. 60541A) an IgG1 isotype control. (Lane 2), the antibody recognizes 5-HT2CR as a 55kDa band.

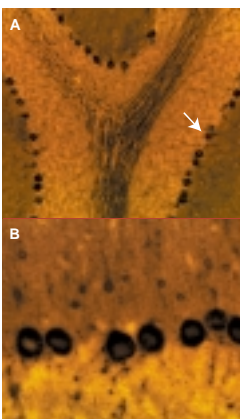


Figure 82. Formalin-fixed frozen adult rat brain sections (cerebellum) stained with the anti GABAB receptor (Cat. No. 60696E). Arrow indicates GABAB Receptor immunoreactivity. Strong immunostaining is seen in the cell bodies and dendrites of Purkinje cells. B is a higher magnification of A.

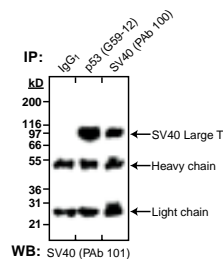
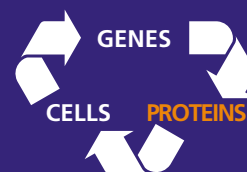


Figure 83. Immunoprecipitation (IP) of large T antigen in SV40-transformed rat ovarian cells (DC3). DC3 cell lysates were immunoprecipitated with antibodies to p53 (G59-12, Cat. no. 14211A), SV40 large T antigen (PAb 100, Cat. no. 14141A) or with an IgG1 isotype control (clone A85-1, Cat. no. 02231D). Proteins were separated by SDS-PAGE and then western blotted (WB) with an antibody recognizing large T antigen (PAb 101, Cat. no. 14111A). The results show that SV40 large T could be immunoprecipitated with anti-large T antibody (PAb 100) or co-immunoprecipitated with an anti-p53 antibody (G59-12).



Antibodies to Rat Cell Biology Markers

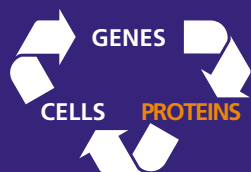
Specificity	Clone	Application	Cat. No.	Format	Size
AKT	polyclonal	WB	68601N	Se	100 µl
AKT (P-Ser473)	polyclonal	WB, IP	68591N	Se	100 µl
Androgen Receptor	G122-25	FC, IHCF, IHCP	15051A	Pu	0.1 mg
Androgen Receptor	G122-77	IP, IHCF	15071A	Pu	0.1 mg
DBP	10	WB	14681A	Pu	0.1 mg
E2F-1	KH95-E2F	WB, IP	14971A	Pu	0.1 mg
FKBP-12	2C1-87	WB, IHC	13521A	Pu	0.1 mg
Jun	G56-206	WB	13411A	Pu	0.1 mg
Jun	IKK-γ	WB	68341A	Pu	0.1 mg
Ku-70/80	162	IP, IF, FC, ELISA	66181A	Pu	0.1 mg
L1 Protein (HPV type 16)	CAMVIR	WB	14481A	Pu	0.1 mg
Latent TGFβ-1 BP	polyclonal	IP, WB, IHCF, IHCP	15866E	Se	100 µl
MyoD	M0Ab5.8A	IP, WB, IHCF	13941A	Pu	0.1 mg
PLC-γ1	polyclonal	IP, WB	15526E	Se	100 µl
PLC-γ2	polyclonal	IP, WB	15996E	Se	100 µl
PKC	MC5	IP, WB, IHCF, IF	14871A	Pu	0.1 mg
SC35	alphaSC35	IP, WB, IF	65201A	Pu	0.1 mg
Sos1	polyclonal	WB, IHC	15191B	Lyo	0.2 mg
Sp1	1C6	WB	13931A	Pu	0.1 mg
Stat1	IF9A7	WB, IP, IF	15221A	Pu	0.1 mg
TCP-1	22B	IP, WB, IF	13321A	Pu	0.1 mg
TCP-1	84A	IP, WB, IF	13311A	Pu	0.1 mg
TCP-1	91A	IP, WB, IF	13331A	Pu	0.1 mg

Antibodies to Rat Tumor Suppressors and Oncogene Markers

Specificity	Clone	Application	Cat. No.	Format	Size
MOM2	SMP14	IP, WB, IHCF, IHCP	65101A	Pu	0.1 mg
p53	G59-12	WB, IP, IHCF, IHCP	14211A	Pu	0.1 mg
p53	PAb 122	WB, IP, IF	14091A	Pu	0.1 mg
p53	PAb 240	WB, IP, IHCF	14461A	Pu	0.1 mg
p53 FITC SET	G59-12, MOPC-21	FC	6732KK	FITC	100 tests
p53 PE SET	G59-12, MOPC-21	FC	6733KK	PE	100 tests
p53 PE SET	DO-7,27-35	FC	6680KK	PE	100 tests
Rb	G3-245	WB, IP, FC, IF, IHCF, IHCP, GS	14001A	Pu	0.1 mg
Rb Cy-Chrome™ Set	G3-245, MOPC-21	FC	6805KK	Cy-Chrome™	100 tests
Rb FITC Set	G3-245, MOPC-21	FC	6684KK	FITC	100 tests
RB PE Set	G3-245, MOPC-21	FC	6685KK	PE	100 tests
SV40 Large T Antigen	PAb-100	IP, IF, IHCF	14141A	Pu	0.1 mg
SV40 Large T Antigen	PAb-101	WB, IP, IF, IHCF	14111A	Pu	0.1 mg

Antibodies to Rat Cell Cycle Markers

Specificity	Clone	Application	Cat. No.	Format	Size
Cdk4	DCS-35	WB, IP	68941A	Pu	0.1 mg
Cyclin A	BF683	WB, IP	14531A	Pu	0.1 mg
		WB, IP	14531C	Pu	0.25 mg
		FC	1370KK	FITC Set	100 tests
Cyclin D1	G124-3216	WB, IP	14561A	Pu	0.1 mg
		WB, IP	14561C	Pu	0.1 mg
		FC	1372KK	FITC Set	100 tests
Cyclin D1	DCS-6	WB, IP	66271A	Pu	0.1 mg

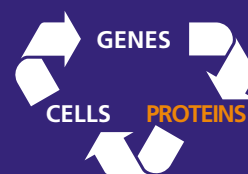


Antibodies to Rat Cell Cycle Markers, *continued*

Specificity	Clone	Application	Cat. No.	Format	Size
Cyclin D3	G107-565	WB, IP	14781A	Pu	0.1 mg
		WB, IP	14781C	Pu	0.25 mg
		FC	1374KK	FITC Set	100 tests
Cyclins D1/D2/D3	G124-259	WB, IP	14841A	Pu	0.1 mg
		WB, IP	14841C	Pu	0.25 mg
		FC	1375KK	FITC Set	100 tests

Antibodies to Rat Neurobiology Markers

Specificity	Clone	Application	Cat. No.	Format	Size
14-3-3	8C3	WB, IP	68291A	Pu	0.1 mg
Dopamine	DBH-41	WB	60101A	Pu	0.1 mg
β -Hydroxylase					
GABA B Receptor	polyclonal	WB, IHCF	60696E	Se	100 μ l
GFAP (cocktail)	4A11, 1B4,2E1	WB, IF,	60341D	Pu	0.5 mg
		IHCF, IHCP			
GFAP	4A11	WB, IF, IHC	60311D	Pu	0.5 mg
GFAP	1B4	WB, IF, IHC	60321D	Pu	0.5 mg
GFAP	20	WB, IF, IHC	60331D	Pu	0.5 mg
Glutamate R (GluR2,R3)	polyclonal	WB, IP, IHCF	60656N	Pu	0.05 mg
Glutamate R (GluR4)	polyclonal	WB, IP	60666N	Pu	0.05 mg
Glutamate R (GluR 5,6,7)	3A11	WB, IF, IHC	60011A	Pu	0.1 mg
Glutamate R (NMDAR1)	54.1	WB, IHC	60021A	Pu	0.1 mg
MAP2 (a+b)	Ap20	WB, IHCF	60171A	Pu	0.1 mg
MASH1	24B7.2D11	WB	68271A	Pu	0.1 mg
Myelin Basic Protein	Myelin BP	WB	69151A	Pu	0.1 mg
Myogenin	F5D	WB, GS, IF, IHCF, IHCP	65121A	Pu	0.1 mg
NF-YA	YA-1a	WB, GS	65131A	Pu	0.1 mg
NCAM	12F8	WB, IHCF	60221A	Pu	0.1 mg
Pan N-CAM	12F11	WB, IHCF	60201A	Pu	0.1 mg
Pan N-CAM	N-CAM 13	WB, IHCF	60211A	Pu	0.1 mg
β Nerve Growth Factor	NGF30	WB, IHCF	60111A	Pu	0.1 mg
Nestin (Glutamate Receptor)	Rat 401	WB, FC, IF, IHCF	60051A	Pu	0.1 mg
NMDAR1	54.1	WB	60021A	Pu	0.1 mg
NMDAR1 (C2') (Glutamate Receptor)	polyclonal	WB	69041G	Se	.25 mg
NMDAR1 (C1) (Glutamate Receptor)	polyclonal	WB	69021G	Se	.25 mg
NMDAR1 (C2) (Glutamate Receptor)	polyclonal	WB	69031G	Se	.25 mg
NMDAR1 (N1) (Glutamate Receptor)	polyclonal	WB	69051G	Se	.25 mg
Tau (Tau-2 Epitope)	Tau-2	WB, IHCF, IHCP	60151A	Pu	0.1 mg
(k) Opiod Receptor (N-terminus)	polyclonal	WB, IHCP	60711A	Pu	0.1 mg
(k) Opiod Receptor (internal region)	polyclonal	WB	60721A	Pu	0.1 mg
(k) Opiod Receptor (N-terminus)	KA8	IP, IHC, EIA	60501A	Pu	0.1 mg
Serotonin	YC5/45	IHCF	60121A	Pu	0.1 mg
Serotonin Receptor (5-HT2AR)	G186-1117	WB, IHCF	60231A	Pu	0.1 mg
Serotonin Receptor (5-HT2BR)	A72-1	WB, IHCF	60531A	Pu	0.1 mg
Serotonin Receptor (5-HT2CR)	A4-2	WB, IHCF	60541A	Pu	0.1 mg



Antibodies to Rat Neurobiology Markers, *continued*

Specificity	Clone	Application	Cat. No.	Format	Size
Somatostatin	YC7	IHCF	60131A	Pu	0.1 mg
Substance P	NC1/34	RIA, IHCF	60091A	Pu	0.1 mg
TrkA	polyclonal	IHCF	68266E	Se	100 µl
Tau (Tau-5 Epitope)	Tau-5	WB, IHCF, IHCP	60161A	Pu	0.1 mg
β-Tubulin	5H1	WB, IF	60181A	Pu	0.1 mg
TRH, TYH, and PAH	PH8	WB, IP, IHCF, IHCP	60061D	Pu	0.5 mg
Hydroxylases					
Tyrosine Hydroxylase	TOH A1	IHCF	60081A	Pu	0.1 mg
VAChT	polyclonal	IHCF	60596E	Se	100 µl

Antibodies to Apoptosis Markers

Specificity	Clone	Application	Cat. No.	Format	Size
Bak	polyclonal	WB	65606E	Se	100 µl
Bax	6A7	WB, IP	66241A	Pu	0.1 mg
Bax	polyclonal	WB, IP, IHCF, IHCP	13686E	Se	100 µl
Bcl-2	polyclonal	WB, IP, IHCP	13456E	Se	100 µl
Bcl-2	polyclonal	WB, IP, IHCP	15616E	Se	100 µl
Bcl-XL	2H12	WB	66461A	Pu	0.1 mg
Bim	polyclonal	WB	68871N	Se	100 µl
E2F-1	KH95/E2F	14971A	14971A	Pu	0.1 mg
Caspase-1	B24-1	WB	66441A	Pu	0.1 mg
Caspase-2	G310-1248	WB	13951A	Pu	0.1 mg
Caspase-4	B25-1	WB	66171A	Pu	0.1 mg
Cytochrome c	6H2.B4	IP	65971A	Pu	0.1 mg
Cytochrome c	7H8.2C12	WB	65981A	Pu	0.1 mg
PARP	7D3-6	WB	66391A	Pu	0.1 mg
PARP	C-210	WB, IF	65196E	Asc	100 µl
Raf	URP26K	IP, WB	13981A	Pu	0.1 mg
Bak	polyclonal	WB, IHCP	65606E	Pu	100 µl



BD RiboQuant™ Ribonuclease Protection Assay (RPA) Systems

The ribonuclease protection assay (RPA) is a highly sensitive and specific method to detect and quantitate RNA. The method is based on the hybridization of target RNA to ³²P-labeled anti-sense RNA probes that have been *in vitro*-transcribed from a DNA template. RNase treatment follows, resulting in degradation of single-stranded RNA and excess probe. The probe and target RNA are resolved by denaturing polyacrylamide gel electrophoresis and the "protected" probe is visualized using autoradiography or phosphorimaging equipment.

BD PharMingen's Multi-Probe Template Sets are composed of a series of biologically related templates, each of distinct length and each representing a sequence in a distinct mRNA species.

Each template set is capable of detecting up to 11 unique gene transcripts in addition to two housekeeping genes, L32 and GAPDH. Samples of total RNA from various sources analyzed for distinct mRNA species using BD PharMingen's RiboQuant™ Multi-Probe Ribonuclease Protection Assay System. The autoradiograms show the probe sets not treated with RNases (Lanes 1) along with the corresponding RNase-protected probes following hybridization with yeast tRNA (2 mg) (Lanes 2) and selected rat RNA (Lanes 3, 4, 5). Note that each undigested control probe band (Lanes 1) migrates more slowly than its protected and digested counterpart (Lanes 3); this is due to the digestion of the flanking sequences in the probe that are not protected by the target mRNA. (Figures 84-88)

Rat RiboQuant™ Products

Description	Size	Cat. No.
BD RiboQuant™ RPA Starter Package	10 rxns	45024K
BD RiboQuant™ <i>In Vitro</i> Transcription Kit	25 rxns	45004K
BD RiboQuant™ RNase Protection Assay Kit	200 rxns	45014K
RNA Isolation Kit		45520K

Rat Cytokine Template Sets

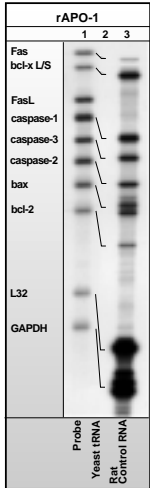
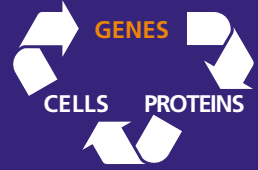
rCK-1	Rat Cytokine Template Set	10 rxns	45027P
rCK-2	Rat Cytokine Template Set	10 rxns	45630P
rCK-3	Rat Cytokine Template Set	10 rxns	45631P

Rat Neurobiology Template Sets

rNT-1	Rat Neurotrophin Template Set		45028P
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Rat Apoptosis Template Sets

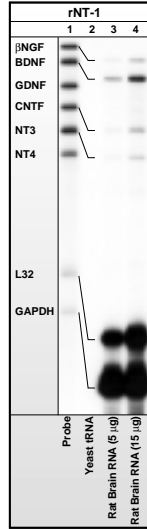
rAPO-1	RiboQuant™ Rat Apoptosis Template Set		45601P
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rAPO-1 (Cat. No. 45601P)

Template	Probe	Protected
FAS	435	406
bcl-X (L)	393	363 (L)
bcl-X (S)	393	325 (S)
FASL	315	286
caspase-1	282	253
caspase-3	255	226
caspase-2	231	202 (L)
bax	210	181
bcl-2	189	160
caspase-2	231	137 (S)
L32	141	112
GAPDH	126	97

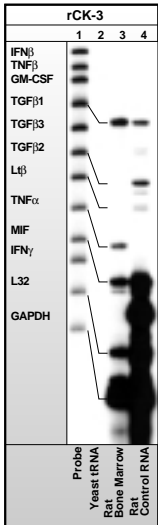
Figure 84



rNT-1 (Cat. No. 45028P)

Template	Probe	Protected
β NGF	351	322
BDNF	315	286
GDNF	282	253
CNTF	255	226
NT3	231	202
NT4	213	184
L32	141	112
GAPDH	126	97

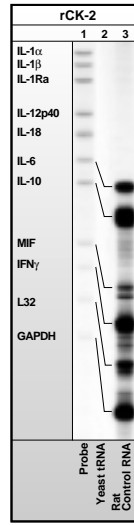
Figure 85



rCK-3 (Cat. No. 45631P)

Template	Probe	Protected
IFN β	390	361
TNF β	351	322
GM-CSF	324	295
TGF β 1	285	256
TGF β 3	255	226
TGF β 2	231	202
Lt β	210	181
TNF α	189	160
MIF	171	142
IFN γ	158	127
L32	141	112
GAPDH	126	97

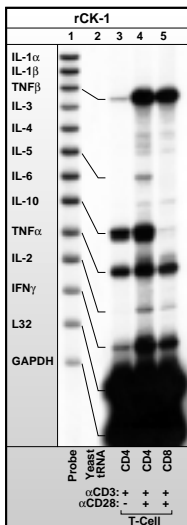
Figure 86



rCK-2 (Cat. No. 45630P)

Template	Probe	Protected
IL-1 α	432	403
IL-1 β	390	361
IL-1Ra	351	322
IL-12p40	285	256
IL-18	255	226
IL-6	231	202
IGIF/IL-18	255	226
IL-10	210	181
MIF	171	142
IFN γ	158	127
L32	141	112
GAPDH	126	97

Figure 87



rCK-1 (Cat. No. 45027P)

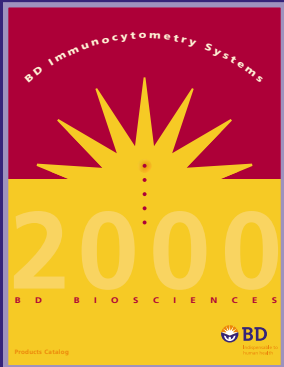
Template	Probe	Protected
IL-1 α	432	403
IL-1 β	390	361
TNF β	351	322
IL-3	315	286
IL-4	285	256
IL-5	255	226
IL-6	231	202
IL-10	210	181
TNF α	189	160
IL-2	171	142
IFN γ	156	127
L32	141	112
GAPDH	126	97

Figure 88

Special thanks to Dr. Frans Kroese for his technical contribution to the development of this brochure.

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