Technical Data Sheet

BUV737 Hamster Anti-Mouse CD48

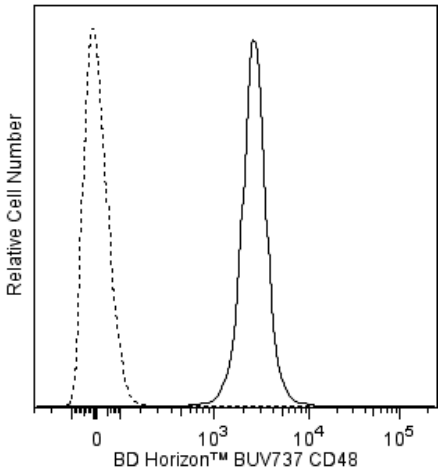
Product Information

Material Number:	**565240**
Alternate Name:	BLAST; BLAST-1; BCM1; HM48-1; MEM-102; Sgp-60; SLAMF2
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	HM48-1
Immunogen:	Mouse T lymphoma MBL-2
Isotype:	Armenian Hamster IgG1, λ3
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The HM48-1 monoclonal antibody specifically binds to CD48 (previously known as BCM1 in mice, Blast-1 in human, and OX-45 in the rat), a GPI-anchored member of the Ig superfamily. It is widely distributed on leukocytes, but not on non-hematopoietic cells, and its ligands include CD2 (LFA-2) and CD244 (2B4 antigen). The HM48-1 mAb blocks binding of soluble CD2 to CD48-bearing cells, blocks the interaction of CD2 and CD244, inhibits spleen cell proliferative responses to mitogens, augments the proliferative response of spleen cells when cross-linked with anti-CD3e mAbs, and inhibits priming of CTL in vitro. In vivo administration of HM48-1 antibody can prolong survival of cardiac allografts, an effect which is greatly enhanced by the addition of anti-CD2 mAb 12-15. This hamster mAb to a mouse leukocyte antigen does not cross-react with rat leukocytes.

The antibody was conjugated to BD Horizon BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



Flow cytometric analysis of CD48 expression on mouse splenocytes. *Mouse splenic leukocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with either BD Horizon™ BUV737 Hamster IgG1, λ1 Isotype Control (Cat. No. 564682; dashed line histogram) or BD Horizon BUV737 Hamster Anti-Mouse CD48 antibody (Cat. No. 565240; solid line histogram). Flow cytometric histograms were derived from gated events with the forward and side light-scattering characteristics of viable splenocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometry System.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

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877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

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Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

Suggested Companion Products

Catalog Number	Name	Size	Clone
564682	BUV737 Hamster IgG1, λ1 Isotype Control (to be replaced with 612820)	50 µg	G235-2356
563794	Brilliant Stain Buffer	100 Tests	(none)
555899	Lysing Buffer	100 mL	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Brown MH, Boles K, van der Merwe PA, Kumar V, Mathew PA, Barclay AN. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J Exp Med.* 1998; 188(11):2083-2090. (Biology)

Kato K, Koyanagi M, Okada H, et al. CD48 is a counter-receptor for mouse CD2 and is involved in T cell activation. *J Exp Med.* 1992; 176(5):1241-1249. (Immunogen: Blocking, (Co)-stimulation, ELISA, Immunoprecipitation, Inhibition, Stimulation, Western blot)

Latchman Y, McKay PF, Reiser H. Identification of the 2B4 molecule as a counter-receptor for CD48. *J Immunol.* 1998; 161(11):5809-5812. (Biology)

Qin L, Chavin KD, Lin J, Yagita H, Bromberg JS. Anti-CD2 receptor and anti-CD2 ligand (CD48) antibodies synergize to prolong allograft survival. *J Exp Med.* 1994; 179(1):341-346. (Biology)

Wong YW, Williams AF, Kingsmore SF, Seldin MF. Structure, expression, and genetic linkage of the mouse BCM1 (OX45 or Blast-1) antigen. Evidence for genetic duplication giving rise to the BCM1 region on mouse chromosome 1 and the CD2/LFA3 region on mouse chromosome 3. *J Exp Med.* 1990; 171(6):2115-2130. (Biology)