Technical Data Sheet

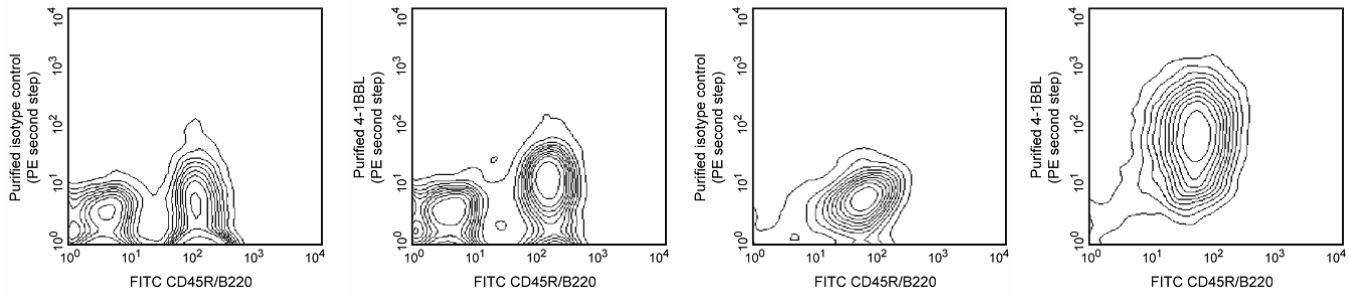
Purified Rat Anti-Mouse 4-1BB Ligand

Product Information

Material Number:	**550532**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	TKS-1
Immunogen:	BALB/c mouse B lymphoma line 2PK-3
Isotype:	Rat (SD) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The TKS-1 antibody reacts with 4-1BB Ligand (4-1BBL), a 97kDa member of the NGF/TNF superfamily which is present on antigen-presenting cells and activated B lymphocytes. 4-1BBL interacts with 4-1BB (CDw137), found predominantly on activated T lymphocytes. This ligand-receptor pair is grouped with pairs such as CD27/CD70, CD30/CD153, OX-40/OX-40L, CD80/CD28, and CD86/CD28, which costimulate T-cell proliferation and cytokine production. The interaction of 4-1BB with 4-1BBL may be reciprocally costimulatory in that 4-1BBL may deliver proliferative signals to B lymphocytes activated by anti-IgM. The TKS-1 mAb stains B cells activated for three days with anti-IgM polyclonal antibody, consistent with an earlier report that soluble 4-1BB-alkaline phosphatase fusion protein binds to anti-IgM-activated B cells. Furthermore, 4-1BBL mRNA has been detected in activated T lymphocytes and activated macrophages. The TKS-1 mAb has been reported to inhibit the binding of soluble 4-1BB to 4-1BBL transfectants and block the *in vitro* costimulation of T-lymphocyte proliferation by 4-1BBL transfectants.



Expression of 4-1BB Ligand (4-1BBL) on mouse splenocytes. *C57BL/6 splenocytes, freshly isolated (left two panels) and following 3-day activation with soluble anti-mouse IgM (Jackson Immunoresearch, right two panels), were stained with purified rat IgG2a, κ isotype control mAb R35-95 (Cat. no. 553927, far left and center right panels) or purified mAb TKS-1 (center left and far right panels), followed by PE-conjugated goat anti-rat Ig (Cat. no. 550767, all panels), then FITC-conjugated anti-mouse CD45R/B220 mAb RA3-6B2 (Cat. no. 553087/553088, all panels). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

Application Notes

Application

Flow cytometry	Routinely Tested
Blocking	Reported

Recommended Assay Procedure:

Because of the low density expression of 4-1BBL on activated B lymphocytes, we recommend the use of a bright second-step reagent, such as PE-conjugated anti-rat Ig polyclonal antibody (Cat. No. 550767). Other reported applications include the blocking of the functional interaction of 4-1BBL with 4-1BB.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
550767	PE Goat Anti-Rat Ig	0.2 mg	Polyclonal
553927	Purified Rat IgG2a, κ Isotype Control	0.5 mg	R35-95
553087	FITC Rat Anti-Mouse CD45R/B220	0.1 mg	RA3-6B2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References

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DeBenedette MA, Shahinian A, Mak TW, Watts TH. Costimulation of CD28- T lymphocytes by 4-1BB ligand. *J Immunol.* 1997; 158(2):551-559.(Biology)

Goodwin RG, Din WS, Davis-Smith T, Anderson DM, Gimpel SD, Sato TA, Maliszewski CR, Brannan CI, Copeland NG, Jenkins NA, et al. Molecular cloning of a ligand for the inducible T cell gene 4-1BB: a member of an emerging family of cytokines with homology to tumor necrosis factor. *Eur J Immunol.* 1993 October; 23(10):2631-2641.(Biology)

Pollok KE, Kim SH, Kwon BS. Regulation of 4-1BB expression by cell-cell interactions and the cytokines, interleukin-2 and interleukin-4. *Eur J Immunol.* 1995; 25(2):488-494.(Biology)

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