

MONOCLONAL ANTIBODY

CD134/OX40

Code No.	Clone	Subclass	Quantity	Concentration
D125-3	W4-3	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: OX40 (CD134/TNFRSF4/ACT35) is a 50 kDa of cell surface glycoprotein. OX40, a member of the tumor necrosis factor (TNF) superfamily, is expressed primarily on activated CD4⁺ T cells. OX40 interacts with OX40 ligand antigen (OX40L, also known as CD252/gp34/CD134L) expressed on activated B cells and antigen presenting cells results in enhanced T cell proliferation and induction of IL-2 production. OX40/OX40L interaction provides a costimulatory signal, resulting in enhanced T cell proliferation and cytokine production. Then, cell proliferation and immunoglobulin secretion in activated B cells are enhanced. OX40/OX40L system mediates the adhesion of activated or HTLV-I-transformed T cells to vascular endothelial cells. TRAF2 and TRAF5 binding to OX40 led to NF-κB activation, and TRAF3 binding appeared to inhibit NF-κB activation

SOURCE: This antibody was purified from C.B-17 SCID mice ascites fluid using protein A agarose. This hybridoma (clone W4-3) was established by fusion of mouse myeloma cell SP2/0 with WKA/H rat splenocyte immunized with the human OX40 transfectant.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with CD134 antigen on Flow cytometry.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; 10-20 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

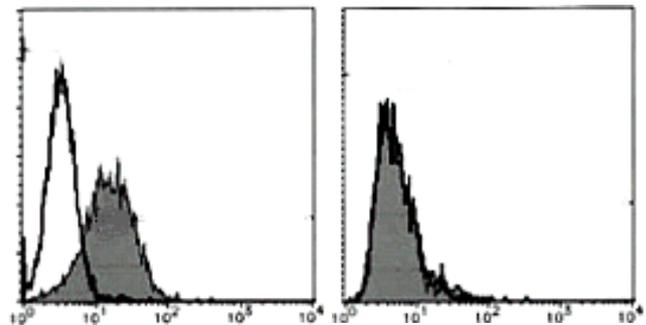
For Research Use Only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	MT2, HPB-MLT	Not Tested	Not Tested
Reactivity on FCM	+		

REFERENCES:

- 1) Kawamata, S., *et al.*, *J. Biol. Chem.* **273**, 5808-5814 (1998)
- 2) Latza, U., *et al.*, *Europ. J. Immun.* **24**, 677-683 (1994)



Flow cytometric analysis of CD134 expression on HPB-MLT (left) and PM1 (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D125-3 to the cells.

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃].
- 2) Resuspend the cells with washing buffer (5 x 10⁶ cells/mL).
- 3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 4) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.

- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 30 µL of 1:40 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; MT2, HPB-MLT)

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|---------|---|
| D057-4 | anti-mouse FasL-FITC (CD178) (FLIM58) |
| D057-6 | anti-mouse FasL-Biotin (CD178) (FLIM58) |
| D069-3 | anti-mouse FasL (CD178) (FLIM4) |
| M031-3 | anti-TRADD (3E11) |
| K0033-3 | anti-DR3 (B65) |
| K0033-4 | anti-DR3-FITC (B65) |
| M081-3 | Rat IgG2a isotype control (2H3) |

RELATED PRODUCTS:

- | | |
|----------|--------------------------------------|
| D113-3 | anti-human TNF α (#1) |
| D114-3 | anti-human TNF β (#1) |
| K0145-3 | CD30 (Ber-H2) |
| K0145-4 | CD30-FITC (Ber-H2) |
| K0039-3 | CD120a/TNFR1 (H398) |
| K0039-4 | CD120a/TNFR1-FITC (H398) |
| K0040-3 | CD120b/TNFR2 (80M2) |
| K0040-4 | CD120b/TNFR2 -FITC (80M2) |
| K0040-5 | CD120b/TNFR2-PE (80M2) |
| K0029-3 | CD137 (4B4-1) |
| K0029-4 | CD137-FITC (4B4-1) |
| K0030-3 | CD137L (5F4) |
| K0030-4 | CD137L-FITC (5F4) |
| D125-3 | CD134/OX40 (W4-3) |
| D126-3 | CD252/OX40L (TAG-34) |
| D051-3 | CD154 (5F3) |
| D051-4 | CD154-FITC (5F2) |
| D200-3 | CD257/BAFF/BLyS (1D6) |
| D200-4 | CD257/BAFF/BLyS-FITC (1D6) |
| D201-3 | CD268/BAFFR/BR3 (8A7) |
| D201-4 | CD268/BAFFR/BR3-FITC (8A7) |
| D201-5 | CD268/BAFFR/BR3-PE (8A7) |
| D267-3 | CD267/TACI (11H3) |
| D267-5 | CD267/TACI-PE (11H3) |
| D222-3 | anti-mouse GITR (DTA-1) |
| D222-3M2 | anti-GITR (DTA-1)(functional grade) |
| D222-4 | anti-mouse GITR-FITC (DTA-1) |
| D222-5 | anti-mouse GITR-FITC (DTA-1) |
| K0031-3 | anti-HVEM (122) |
| K0031-4 | anti-HVEM-FITC (122) |
| MD-10-3 | anti-human Fas (CD95) (UB2) |
| MD-10-4 | anti-human Fas-FITC (CD95) (UB2) |
| MD-10-5 | anti-human Fas-PE (CD95) (UB2) |
| MD-11-3 | anti-human Fas (CD95) (ZB4) |
| SY-001 | anti-human Fas (CD95) (CH-11) |
| D041-3 | anti-human FasL (CD178) (4H9) |
| D041-4 | anti-human FasL-FITC (CD178) (4H9) |
| D041-5 | anti-human FasL-PE (CD178) (4H9) |
| D041-6 | anti-human FasL-Biotin (CD178) (4H9) |
| D042-3 | anti-human FasL (CD178) (4A5) |
| D026-3 | anti-mouse Fas (CD95) (RMF2) |
| D027-3 | anti-mouse Fas (CD95) (RMF6) |
| D057-3 | anti-mouse FasL (CD178) (FLIM58) |