

MONOCLONAL ANTIBODY

CD252/OX40L

Code No.	Clone	Subclass	Quantity	Concentration
D126-3	TAG-34	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: OX40 ligand (OX40L, also known as CD252/gp34/CD134L) is a 34 kDa of type II membrane protein. OX40L, a member of the tumor necrosis factor (TNF) superfamily, is expressed on activated B cells and antigen presenting cells. OX40L interacts with OX40 antigen (CD134/TNFRSF4/ACT35) expressed predominantly on activated T cells to increase proliferation and 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. Human T-cell leukemia virus type 1 (HTLV-1) was the first human retrovirus to be characterized, and it is implicated in the pathogenesis of adult T-cell leukemia (ATL). It is known that HTLV-1-infected cells express various costimulatory molecules such as CD80, CD86, CD40 and OX40L.

SOURCE: This antibody was purified from C.B-17 SCID mouse ascites fluid by ammonium sulfate precipitation followed by gel filtration through Superdex 200 in PBS. This hybridoma (clone TAG-34) was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with glycoproteins collected from the HTLV-1 virus-depleted MT2 cell culture supernatant on a lentil-lectin column.

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 human CD252 antigen on Flow cytometry.

APPLICATIONS:

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

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	MT2, PM1	Not Tested	Not Tested
Reactivity on FCM	+		

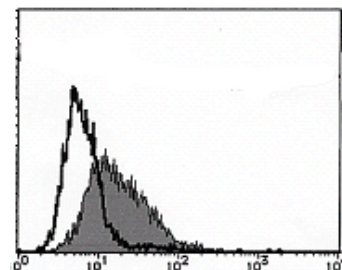
INTENDED USE:

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

REFERENCES:

- 1) Biagi, E., *et al.*, *Blood* **105**, 2436-2442 (2005)
- 2) Hanna, J., *et al.*, *J. Immunol.* **173**, 6547-6563 (2004)
- 3) Kashiwakura, J., *et al.*, *J. Immunol.* **173**, 5247-5257 (2004)
- 4) Wang, Q., *et al.*, *Tissue Antigens* **64**, 566-574 (2004)
- 5) Takahashi, Y., *et al.*, *J. Virol.* **75**, 6748-6757 (2001)
- 6) Tanaka, Y., *et al.*, *Int. J. Cancer* **36**, 549-555 (1985)

Clone TAG-34 is used in these references.



Flow cytometric analysis of CD252 expression on PM1. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D126-3 to the cells.

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step 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 (5x10⁶ 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 10 μ L of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 40 μ L of the primary antibody at the concentration of as suggest 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 μ L of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) 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 μ L of the washing buffer and analyze by a flow cytometer.

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)
D057-4	FITC labeled Anti-mouse FasL (CD178) (FLIM58)
D057-6	Biotin labeled Anti-mouse FasL (CD178) (FLIM58)
D069-3	Anti-mouse FasL (CD178) (FLIM4)
M031-3	Anti-TRADD (3E11)
K0033-3	Anti-DR3 (B65)
K0033-4	FITC labeled Anti-DR3 (B65)

(Positive control for Flow cytometry; PM1)

RELATED PRODUCTS:

- D113-3 Anti-human $\text{TNF}\alpha$ (#1)
- D114-3 Anti-human $\text{TNF}\beta$ (#1)
- K0039-3 CD120a/TNFR1 (H398)
- K0039-4 FITC labeled CD120a/TNFR1 (H398)
- K0040-3 CD120b/TNFR2 (80M2)
- K0040-4 FITC labeled CD120b/TNFR2 (80M2)
- K0040-5 PE labeled CD120b/TNFR2 (80M2)
- K0145-3 CD30 (Ber-H2)
- K0145-4 FITC labeled CD30 (Ber-H2)
- K0029-3 CD137 (4B4-1)
- K0029-4 FITC labeled CD137 (4B4-1)
- K0030-3 CD137L (5F4)
- K0030-4 FITC labeled CD137L (5F4)
- D125-3 CD134/OX40 (W4-3)
- D051-3 CD154 (5F3)
- D051-4 FITC labeled CD154 (5F2)
- D200-3 CD257/BAFF/BLyS (1D6)
- D200-4 FITC labeled CD257/BAFF/BLyS (1D6)
- D201-3 CD268/BAFFR/BR3 (8A7)
- D201-4 FITC labeled CD268/BAFFR/BR3 (8A7)
- D222-3 Anti-mouse GITR (DTA-1)
- D222-4 FITC labeled anti-mouse GITR (DTA-1)
- D222-5 PE labeled anti-mouse GITR (DTA-1)
- K0031-3 Anti-HVEM (122)
- K0031-4 FITC labeled Anti-HVEM (122)
- MD-10-3 Anti-human Fas (CD95) (UB2)
- MD-10-4 FITC labeled Anti-human Fas (CD95) (UB2)
- MD-10-5 PE labeled Anti-human Fas (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 FITC labeled Anti-human FasL (CD178) (4H9)
- D041-5 PE labeled Anti-human FasL (CD178) (4H9)
- D041-6 Biotin labeled Anti-human FasL (CD178) (4H9)