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

MONOCLONAL ANTIBODY

Anti-HVEM

Code No.	Clone	Subclass	Quantity	Concentration
K0031-3	122	Mouse IgG1 κ	100 μg	1 mg/mL

BACKGROUND: Herpes virus entry mediator (HVEM) is a member of the tumor necrosis factor receptor (TNFR) superfamily that has a role in herpes simplex virus entry, in T cell activation and in tumor immunity. HVEM is constitutively expressed on $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD14^+$ monocytes, neutrophils and dendritic cells (DCs). The cytoplasmic region of HVEM binds to several members of the TNFR-associated factor (TRAF) family, namely, TRAF1, TRAF2, TRAF3 and TRF5, but not to TRAF4 or TRAF6, and that it activates transcription factors NF-κB and AP-1. The co-expression of glycoprotein D (gD) and HVEM results in the inhibition of the NF-κB activation that is induced by the HVEM overexpression.

SOURCE: This antibody was purified from hybridoma (clone 122) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with Balb/c mouse splenocyte immunized with the recombinant HVEM.

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 HVEM 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 **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	THP-1	Not Tested	Not Tested
Reactivity on FCM	+		

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Compaan, D. M., *et al.*, *J. Biol. Chem.* **280**, 39553-39561 (2005)
2) Gonzalez, L. C., *et al.*, *PNAS* **102**, 1116-1121 (2005)
3) Jung, H.W., *et al.*, *Exp. Mol. Med.* **35**, 501-508 (2003)
4) La, S., *et al.*, *Mol. Cells* **14**, 398-403 (2002)
5) Tamada, K., *et al.*, *J.Immunol.* **164**, 4105-4110 (2000)
6) Zhai, Y., *et al.*, *J. Clin. Invest.* **102**, 1142-1151 (1998)
7) Kwon, B.S., *et al.*, *J. Biol. Chem.* **272**, 14272-14276 (1997)

Clone 122 is used in the reference 1) - 3).

RELATED PRODUCTS:

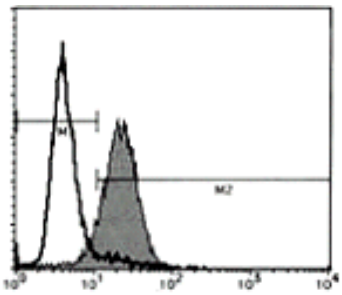
K0031-4	FITC labeled Anti-HVEM (122)
K0029-3	CD137 (4B4-1)
K0030-3	CD137L (5F4)
K0030-4	FITC labeled CD137L (5F4)
K0039-3	Anti-TNF-R1 (H398)
K0039-4	FITC labeled Anti-TNF-R1 (H398)
K0040-3	Anti-TNF-R2 (80M2)
K0040-4	FITC labeled Anti-TNF-R2 (80M2)
M031-3	Anti-TRADD (3E11)
D113-3	Anti-Human TNF-α (#1)
D114-3	Anti-Human TNF-β (lymphotoxin) (#1)
D125-3	Anti-OX40 (W4-3)
D126-3	Anti-OX40L (TAG-34)
D200-3	Anti-Human BAFF/BlyS (1D6)
D200-4	FITC labeled Anti-Human BAFF/BlyS (1D6)
D201-3	Anti-Human BAFF-R/BR3 (8A7)
D222-3	Anti-GITR (DTA-1)
D222-4	FITC labeled Anti-GITR (DTA-1)
D222-5	PE labeled Anti-GITR (DTA-1)
M028-3	Anti-TRAF1 (3D4)
M112-3	Anti-TRAF2 (6F8)
592	Anti-TRAF2 (poly)
M092-3	Anti-TRAF6 (1F8)
597	Anti-TRAF6 (poly)
4842	IMMUNOCYTO Intracellular TNF-α Detection Kit

PROTOCOLS:
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% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

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 µL 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 µL of anti-HVEM (122) (25 µg/mL) diluted with 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-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 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; THP-1)



Flow cytometric analysis of HVEM expression on THP-1 cells. *Open histogram indicates the reaction of Isotypic control to the cells. Shaded histogram indicates the reaction of K0031-3 to the cells.*

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all step described below.

1) Add 20 µL of anti-HVEM monoclonal antibody (122) (50 µg/mL) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.