

MONOCLONAL ANTIBODY

Anti-CTLA-4/CD152

Code No.	Clone	Subclass	Quantity	Concentration
D091-3	MIH8	Mouse IgG3 κ	100 μ g	1 mg/mL

BACKGROUND: The coreceptor cytotoxic T lymphocyte-associated antigen 4 (CTLA-4/CD152) is a member of the immunoglobulin superfamily, expressed on activated T lymphocytes 2-3 days after stimulation via the T cell receptor, regulate the threshold of signals during T cell activation, but the mechanism is still not clear. Like CD28, CTLA-4 shares the same ligands, namely CD80 (B7-1) and CD86 (B7-2), but CTLA-4 binds with an affinity 10- to 20-fold higher than that of CD28. Mutation of CTLA-4 gene has been related to autoimmune diseases such as insulin-dependent diabetes mellitus, Graves disease, Hashimoto thyroiditis, celiac disease, systemic lupus erythematosus.

SOURCE: This antibody was purified from hybridoma (clone MIH8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with DBA/2 mouse splenocyte immunized with full-length human CTLA-4 transfected p815 cells.

FORMULATION: 100 μ g IgG in 100 μ L 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 CTLA-4 on Flow cytometry.

APPLICATIONS:

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

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

INTENDED USE:

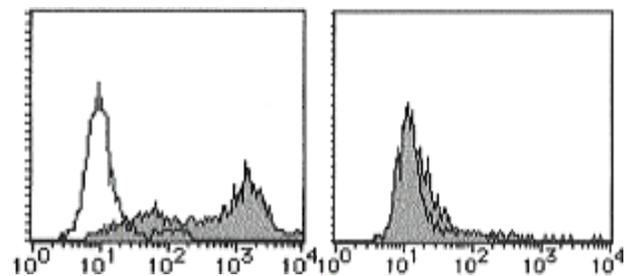
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

- 1) Dong, H., *et al.*, *Nat. Med.* **5**, 1365-1369 (1999)
- 2) Saito, K., *et al.*, *J. Immunol.* **160**, 4225-4231 (1998)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	transfectant	Not Tested	Not Tested
Reactivity on FCM	+		



Flow cytometric analysis of CTLA-4/CD152 expression on transfectant (left) and parental cells (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D091-3 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 μ L 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 μ L 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 20 μ L 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 4°C.
- 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. 238) diluted with the washing buffer. Mix well and incubate for 30 minutes at 4°C.

- 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.

RELATED PRODUCTS:

D091-6	anti-CTLA4/CD152-Biotin (MIH8)
D222-3	anti-mouse GITR (DTA-1)
D222-3M2	anti-mouse GITR (DTA-1) (functional grade)
D222-4	anti-mouse GITR-FITC (DTA-1)
D222-5	anti-mouse GITR-PE (DTA-1)
D239-3M2	anti-mouse FR4 (TH6) (functional grade)
D239-3	anti-mouse FR4 (TH6)
D239-4	anti-mouse FR4-FITC (TH6)
D239-5	anti-mouse FR4-PE (TH6)
D239-6	anti-mouse FR4-Biotin (TH6)
PM024	anti-mouse Foxp3 (poly)
D237-3	anti-mouse Foxp3 (MF-14)
D237-4	anti-mouse Foxp3-FITC (MF-14)
D237-6	anti-mouse Foxp3-Biotin (MF-14)
M120-3	anti-Foxp3 (99D04)
M078-3	Mouse IgG3 isotype control (6A3)