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

MONOCLONAL ANTIBODY

# FITC labeled Anti-TNF-R1

| Code No. | Clone | Subclass | Quantity | Concentration |
|---|---|---|---|---|
| K0039-4 | H398 | Mouse IgG2a | 1 mL | 50 μg/mL |

**SOURCE:** This antibody was concentrated from hybridoma (clone H398) supernatant. This hybridoma was established by fusion of mouse myeloma cell NSO with Balb/c mouse splenocyte immunized with HL60 derived affinity-purified TNF receptor material.

**FORMULATION:** 50 μg IgG in 1 mL volume of PBS containing 1% BSA and 0.09% $NaN_3$.
*Azide may react with copper lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

**REACTIVITY:** This antibody reacts with human TNF-R1 on Flow cytometry.

**APPLICATIONS:**
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow Cytometry; 20 μg / mL (final concentration)

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

**SPECIES CROSS REACTIVITY:**

| Species | Human | Mouse | Rat |
|---|---|---|---|
| Cell | U937 | Not Tested | Not Tested |
| Reactivity on FCM | + | | |

**INTENDED USE:**
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**REFERENCE:**
1) Thoma, B., *et al., J. Exp. Med.* **172**, 1019-1023 (1990)

Clone H398 is used in this reference.

**RELATED PRODUCT:**
K0039-3 Anti-TNF-R1 (H398)

**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% $NaN_3$].
2) Resuspend the cells with washing buffer ($5x10^6$ 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 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 the FITC labeled anti-TNF-R1 monoclonal antibody (H398) (50 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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for flow cytometry; U937)

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