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

MONOCLONAL ANTIBODY

# Anti-RCAS1 (Human) mAb

| Code No. | Clone | Subclass | Quantity | Concentration |
|---|---|---|---|---|
| D060-3 | 22-1-1 | Mouse IgM | 100 µL | 1 mg/mL |

**BACKGROUND:** RCAS1 (receptor-binding cancer antigen expressed on SiSo cells) is a novel tumor-associated antigen expressed in human uterine and ovarian carcinomas. The predicted amino acid sequence of RCAS1 (213 aa) possesses an N-terminal transmembrane region and a coiled-coil structure in the C-terminal portion, indicating that RCAS1 is a type II membrane protein able to form oligomers through the coiled-coil structure. RCAS1 revealed different expression pattern from the known tumor associated antigens such as, YH206, GA733, CA125, CEA and sialyl Lewis molecules in human tumor cell lines. Recent study indicated RCAS1 acts as a ligand for a putative receptor present on various human cells including T, B and NK cells. RCAS1 inhibited the *in vitro* growth of receptor-expressing cells and induced apoptosis. It was suggested that tumor cells might evade immune surveillance by expression of RCAS1.

**SOURCE:** This antibody was purified from hybridoma (clone 22-1-1) supernatant using protein L agarose. This hybridoma was established by fusion of mouse myeloma cell x63.Ag8.653 with Balb/c mouse splenocyte immunized with human uterine cervical adenocarcinoma cells.

**FORMULATION:** 100 µg IgM in 100 µL volume of PBS containing 50% glycerol and 0.5M NaCl, 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 RCAS1 on Flow cytometry and Immunohistochemistry.

**APPLICATIONS:**

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Reference 1)-3), 5)-7)
Immunocytochemistry; 1-5 µg/mL
Flow cytometry; 1-5 µg/mL (final concentration)

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

**INTENDED USE:**

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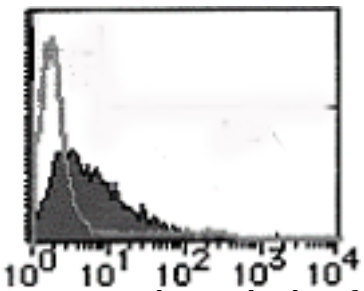
**SPECIES CROSS REACTIVITY:**

| Species | Human | Mouse | Rat |
|---|---|---|---|
| Cell | SiSo | Not tested | Not tested |
| Reactivity on FCM | + | | |

**REFERENCES:**

1) Giaginis, C., *et al*., *Dis Markers.* **35**, 213-219 (2013) [IHC]
2) Giaginis, C., *et al*., *Med Sci Monit.* **18**, BR123-129 (2012) [IHC]
3) Theocharis, S., *et al., Med Sci Monit.* **17**, BR228-234 (2011) [IHC]
4) Wolf, J., *et al.*, *FASEB J*. **24**, 4000-4019 (2010) [FCM]
5) Oshikiri, T., *et al., Clin. Cancer Res.* **12**, 411-416 (2006) [IHC]
6) Reimer, R. A., *et al*., *BMC Cancer* **5**, 47-59 (2005) [IHC]
7) Leelawat, K., *et al*., *J. Clin. Pathol*. **56**, 764-768 (2003) [IHC]
8) Suzuoki, M., *et al*., *Ann. Surg. Oncol*. **9**, 388-393 (2002)
9) Izumi, M., *et al*., *Cancer* **92**, 446-451 (2001)
10) Matsushima, T., *et al*., *Blood* **98**, 313-321 (2001)
11) Nakashima, M., *et al., Nat. Med.* **5**, 938-942 (1999)
12) Sonoda, K., *at al., Cancer* **77**, 1501-1509 (1996)

Clone 22-1-1 is used in these references.




***Flow cytometric analysis of RCAS1 expression on SiSo.*** *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D060-3 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% $NaN_3$].
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 primary antibody at the concentration of as suggest in **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 1:100 FITC conjugated anti-mouse IgM (MBL; code no. IM-0820) 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; SiSo)

**Immunocytochemical staining for paraffin-embedded sections ; SAB method**

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% $H_2O_2$ for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 μL of 30% $H_2O_2$ in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

## RELATED PRODUCTS:

| | |
|---|---|
| D060-3 | Anti-RCAS1 (Human) mAb (22-1-1) |
| D060-3H | Anti-RCAS1 (Human) mAb (22-1-1) |
| M079-3 | Mouse IgM (isotype control) (7E10) |