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Product datasheet for AM26435AF-N

EBAG9 Mouse Monoclonal Antibody [Clone ID: 22-1-1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	22-1-1
Applications:	FC, IHC
Recommended Dilution:	Flow cytometry: 1 - 5 µg/ml. Immunohistochemical staining of paraffin sections: 1 - 5 µg/ml. For details see protocols below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgM
Clonality:	Monoclonal
Immunogen:	Human uterine cervical adenocarcinoma cell
Specificity:	This antibody recognizes RCAS1.
Formulation:	100 ug IgG in 100 uL volukme of PBS containing 50% glycerol and 0.5M NaCl, pH 7.2 No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-L Sepharose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	estrogen receptor binding site associated, antigen, 9
Database Link:	Entrez Gene 9166 Human O00559

View online »

Background: RCAS1 (receptor-binding cancer antigen expressed on SiSo cells) is a novel tumoreassociated antigen expressed in human uterine and ovarian carcinomas. The predicted amino acid sequence of RCAS1 (213 a.a.) 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 tumore associated antigens such as, YH206, GA733, CA125, CEA and sialyl Le molecules in human tumore 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 tumore cells might evade immune surveillance by expression of RCAS1.

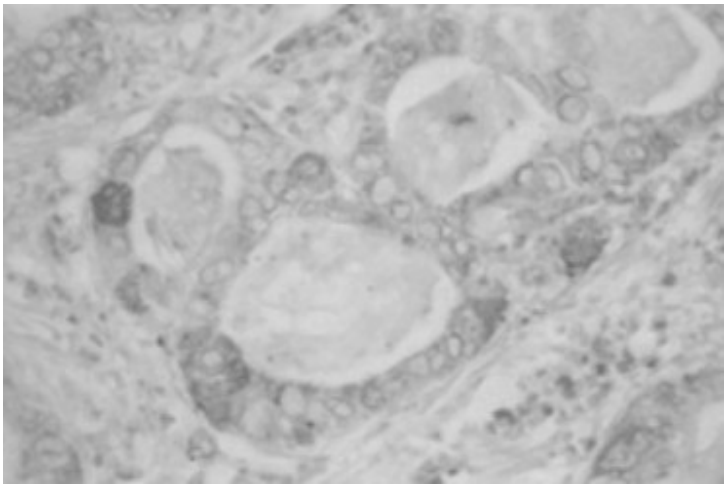
Synonyms: EB9, PDAF

Note: This product was originally produced by MBL International.

Protocol:
Preparation for Flowcytometric analysis
We use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Prepare Wash solution (0.5% BSA, 0.05% NaN3 in PBS) and antibody solutions. Primary antibody solution : 1-5µg/ml of RCAS1 antibody in the Wash solution. Secondary antibody solution : Anti-Mouse IgM conjugated with PE diluted 1:200 with the Wash solution containing 20% of normal goat serum.
2) Wash the cells with Wash solution and suspend 1x10e5 cells with 1 ml of the wash solution.
3) Centrifuge at 3000 rpm for 1 minute at 4 oC. Remove supernatant by careful aspiration.
4) Add 100 µl of ice cold Wash solution containing 40% of normal goat serum. Vortex tube gently and incubate on ice for 15 min.
5) Add 150 µl of the 1st antibody solution. Vortex tube gently and incubate on ice for 30 min.
6) Add 0.7 ml of the Wash solution and centrifuge at 3000 rpm at 4oC for 1 min.
7) Wash the cells with the wash buffer 3 times. Remove supernatant by careful aspiration.
8) Add 150 µl of the 2nd antibody solution. Vortex tube gently and incubate on ice for 30 minutes.
9) Add 0.7 ml of the Wash solution and centrifuge at 3000 rpm at 4oC for 1 min.
10) Wash the cells with the wash buffer 3 times. Remove supernatant by careful aspiration.
11) Add 0.4 ml of the Wash solution and vortex tube gently and analyze as soon as possible in a flowcytometer as the following condition.
CHANNEL DATA MODE Detector lebel Amplifire lebel FSC Linear E00 1.03 SSC Linear 320 1.00 FL2 Log 450 Log
Immunohistochemical staining
For tissue section : ABC method
1) Deparaffinize section, hydrate to water (Xylene-3 times, Ethanol-3 times, PBS 3 times)
2) Wash in PBS for 5 minutes before starting the staining.
3) Remove slides from PBS and cover each section with 100-200 µl of 3% H 2O2 for 10 minutes to block endogenous peroxidase activity. Wash in PBS twice for 5 minutes each.

4) Remove slides from PBS, wipe gently around each section and cover tissues with 100-200µl of Protein Blocking Agent for5 minutes.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with 100-200µl of primary antibody at the concentration of as suggest in APPLICATIONS (The concentration of antibody to be used will depend on several variables and the abundance of the antigen) in Protein Blocking Agent.
6) Incubate the section for 1 hour at room temperature.
7) Wash the slide by gentle removing antibody with a stream from a wash bottle or pipet containing buffer : do not hit tissue section. Wash 3 times with PBS for 5 minutes each.
8) Wipe gently around each section and cover tissues with 100-200 µl of Polyvalent Biotinylated Ab.
9) Incubate for 30 minutes at room temperature.
10) Wash as in 7).
11) Wipe gently around section and cover tissues with 100-200 µl of Streptavidin conjugated HRP.
12) Incubate for 30 minutes at room temperature.
13) Wash as in 7).
14) Visualize with DAB substrate/chromogen (20mg of DAB in 400 ml of PBS containing 40 µl of 30% H2O2) for approximatery 15 minutes. Wash in distilled water.
*DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Counterstain in hematoxylin for 1 minute.
16) Wash the slide by gentle removing hematoxylin with a stream from a wash bottle.
17) Soak the slide in PBS and leave for 5 minutes.
18) Dehydrate the slide by soaking the slide 3 times in Ethanol for 3 minutes each followed by soaking the slide 3 times in Xylene for 3 minutes each.
19) Mounting.

Product images:



IHC on human uterine cervical adenocarcinoma