V.5.0

Anti-Rac1 Monoclonal Antibody
Cat. # ARC03-S

Upon arrival, store at 4°C (dessicated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	25 µl when reconstituted
Validated applications:	WB, IF
Species reactivity:	All

Background Information

Rac1 is one of the most extensively studied members of the Rho GTPase family and is involved in a wide range of cellular responses, including cytoskeletal reorganization and metastasis. Rac1 is expressed in a large number of different cell types. Most commercially available Rac1 antibodies also recognize one or more of Rac2, Rac3 and Cdc42 (Table 1 and Figure 1)

Material

The anti-Rac1 antibody is a mouse monoclonal antibody that specifically recognizes Rac1. Extensive Quality Control analysis has shown that the antibody does not recognize Rac2, Rac3, Cdc42 or other small GTPases. ARC03 is supplied as a lyophilized white powder.

	Rac1	Rac2	Rac3	Cdc42
Cytoskeleton, Inc. (Cat# ARC03)	**Yes**	**No**	**No**	**No**
Upstate (Cat.# 05-389)	Yes	No	Yes	No
Santa Cruz (Cat# sc-95)	Yes	No	Yes	Yes
BD Biosciences (Cat# 610550)	Yes	Yes	Yes	Yes

Table 1. Comparison of several commercial available Rac1 antibodies. The Rac1 antibodies from BD biosciences (Cat.# 610650), Upstate (Cat. # 05-389) and Santa Cruz (Cat. # SC-95) were compared with Cytoskeleton Rac1 antibody (ARC03) for their Rac1 specificity. Yes = cross-reacts; No = No crossreactivity.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube . Reconstitute to 500 µg/ml by resuspending in 25 µl of Milli-Q water and store at 4°C for six months (gentamicin sulfate (50 µg/ml) or other antimicrobial can also be added). THE ANTIBODY SHOULD NOT BE FROZEN.

Methods

Western blot analysis

Reagents:

1) Anti-Rac1 (Cat. # ARC03)
2) SDS-PAGE and Western blot equipment
3) PVDF or Nitrocellulose membrane (Millipore Inc.)
4) Transfer Buffer (ice cold): 25 mM Tris-HCl, pH 8.3; 192 mM glycine, 15% methanol
5) TBST: 10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20
6) Blotto: 5% non-fat dry milk in TBST
7) HRP-conjugated goat anti-mouse antibody (Jackson labs)
8) Chemiluminescence detection reagents (ECL, Amersham Biosciences)

Method:

1) Separate protein samples on a 4-20% SDS PAGE gel until the dye-front reaches the bottom of the gel.
2) Electroblot the proteins onto PVDF or Nitrocellulose membrane for 45 min at 75V with fresh transfer buffer.
3) Block the membrane in Blotto for 60 min at room temperature.
4) Probe with 1 µg/ml (1:500 dilution) of ARC03 in TBST for 1h.
5) Wash the membrane three times with TBST for 5 min each.
6) Probe with 1:20,000 dilution of the anti-mouse-HRP antibody in TBST for 30 min.
7) Wash the membrane six times with TBST for 5 min each.
8) Process the blots for chemiluminescence detection using a high potency reagent such as ECL.
9) Typical results are shown in Figure 1.

Figure 1. ***Western blot of purified Rac1, 2, 3, Cdc42 and platelet extracts probed with anti-Rac1 monoclonal antibody (Cat. # ARC03).***

The detection of 25 ng His-Rac1 (lane 1), His-Cdc42 (lane 2), 50 µg of platelet extract (lane 3), His-Rac2 (lane 4) and His-Rac3 (lane 5). ARC03 does not cross-react with Rac2, 3 or Cdc42. The blot was probed with a 1 µg/ml (1:500) dilution of ARC03, 30s exposure time.

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Immunocytochemistry

Reagents:

1) Tissue culture cells grown on glass coverslips
2) Anti-Rac1 (Cat. # ARC03)
3) Rhodamine conjugated anti-mouse antibody (Jackson Labs, Inc.)
4) Phosphate Buffered Saline (PBS) pH 7.4
5) Methanol at -20°C
6) Permeabilization Buffer (1% Triton X-100 in PBS)
7) Blocking Buffer (3% BSA in PBS)
8) Polyvinyl alcohol antifade mounting medium with DABCO (Fluka Cat. # 10981)
9) Glass microscope slide (25 x 75 x 1 mm)

Method:

1) Grow tissue culture cells on glass coverslips until semi-confluent.
2) Remove culture media and gently wash the cells once with isotemp PBS (37°C).
3) Fix the cells with methanol at -20°C for 3 min.
4) Wash the cells three times with PBS.
5) Place the coverslips with the cell side up on parafilm inside of a petri dish. Maintain a humid atmosphere by placing a piece of wet filter paper inside the covered petri dish. Add 100 µl of Permeabilization Buffer to each coverslip and incubate for 20 min.
6) Remove Permeabilization Buffer, add 100 µl Block Buffer, and incubate for 30 min.
7) Wash the coverslips once with PBS.
8) Add 200 µl of 25 µg/ml (1:20 dilution) of ARC03 antibody in Blocking Buffer to each coverslip. A 1:10 dilution of antibody can be used for darker staining. Incubate for 1 h.
9) Wash each coverslip three times in Permeabilization Buffer (let stand for 5 min each).
10) Add 200 µl of a 1:500 dilution of rhodamine conjugated anti-mouse antibody in Blocking Buffer to each coverslip. Incubate for 30 min.
11) Wash each coverslip three times in PBS (let stand for 5 min each).
12) Invert the coverslips on a drop of antifade mounting media on a glass slide. Gently remove the excess media with a tissue and allow mounting media to dry.
13) Examine the stained coverslips using a fluoresence microscope equipped with filter sets suitable for rhodamine fluorophores.
14) Store the slides in the dark at 4°C.
15) Typical results of Rac1 staining are shown in Figure 2.

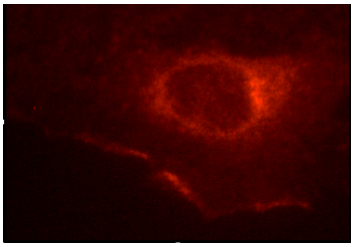

Figure 2. ***Immunofluorescence image of mouse Swiss 3T3 cells stained with Anti-Rac1 monoclonal antibody (Cat. # ARC03)***. Swiss 3T3 cells were grown to semi-confluency and fixed with methanol. Immunofluorescence staining using 1 µg/ml (1:20 dilution) of ARC03 antibody is shown (red). The primary antibody was detected with a 1:500 dilution of goat anti-mouse rhodamine conjugated antibody. Photograph was taken with a 100X objective lens.

Product Uses

- Detection of Rac1 in human, mouse, rat or other extracts

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com