

## Anti-Rac1 Monoclonal Antibody

Cat. # ARC03

Upon arrival, store at 4°C (desiccated)

See datasheet for storage after reconstitution

### 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. Human platelet extract is included as a positive control.

	Rac1	Rac2	Rac3	Cdc42
<b>Cytoskeleton, Inc. (Cat# ARC03)</b>	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-react; 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 100 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.

Resuspend the platelet extract control protein in 500 µl of 1x SDS-PAGE sample loading buffer (63 mM Tris HCL, pH 6.8, 2.3% SDS, 5.0% β-mercaptoethanol, 0.05% bromophenol blue, 10% glycerol) for a final concentration of 2 mg/ml, aliquot into 20 X 25 µl amounts (50 µg each), and store at -70°C.

### 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-Rac1 (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.

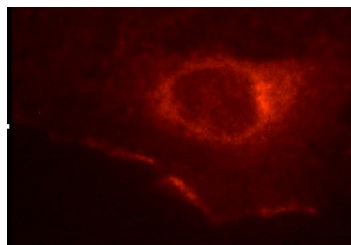
## Immunocytochemistry

### 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 fluorescence 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](http://www.cytoskeleton.com)