ProductInformation

MONOCLONAL ANTI-ACTR (AIB1)
CLONE AX 15
Mouse Ascites Fluid

Product Number **A4469**

Product Description
Monoclonal Anti-ACTR (AIB1) (mouse IgG isotype) is derived from the AX-15 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with a GST fusion protein containing amino acids 605-1294 of human ACTR.

Monoclonal Anti- ACTR (AIB1) recognizes ACTR (150 kD) and another doublet (110 kD) by immunoblotting. Monoclonal Anti-ACTR/AIB1 reacts with human ACTR, other species have not been tested.

Monoclonal Anti- ACTR (AIB1) may be used for the detection of ACTR (AIB1) by immunoblotting and immunoprecipitation.

ACTR, a nuclear receptor coactivator, is also referred to as amplified in breast cancer 1 (AIB1), thyroid hormone receptor activator molecule-1 (TRAM-1), receptor-associated cactivator 3 (RAC3 and SRC3. ACTR is overexpressed in breast and ovarian cancer cell lines. The SRC-1 family of coactivators, of which ACTR is a member, interacts with steroid hormones and enhances transcription. ACTR interacts with estrogen receptors in breast and ovarian cancer cell lines and induces extra transcription. ACTR thus contributes to the development of breast and ovarian cancer when it interacts with estrogen receptors in this way.[1]
In addition to ACTR's cancer involvement it also plays a role in the formation of a multisubunit coactivator complex by gathering together the two nuclear factors CBP and P/CAF.[3]

Reagents
The product is supplied as mouse ascites fluid, containing 30% glycerol and 0.035% sodium azide.

Precautions and Disclaimer
Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability
Store at 0°C to −20°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure
1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 15 µl of Monoclonal Anti-ACTR (AIB1) to 500 µg – 1 mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein G-Agarose beads (50 µl packed beads) (Sigma Product No. P2294).
5. Gently rock reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile
Recommended use: 15 µl of Monoclonal Anti-ACTR (AIB1) will immunoprecipitate ACTR from 0.5-1 mg of a BG-1 ovarian cell RIPA lysate.

Recommended working dilution for immunoblotting is 1:500 of Monoclonal Anti-ACTR (AIB1) a BG-1 ovarian

cell RIPA lysate, anti-Mouse IgG conjugate to Peroxidase and enhanced chemiluminescence.
Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

1. Anzick, S.L., et al., Science, **277**, 965 (1997).
2. Yao, T.P., et al. Proc. Natl. Sci. USA, **93**, 10626 (1996).
3. Chen, H., et al. Cell, **90,** 569 (1997).

jo/lpg 9/99