Affinity-Purified Goat Anti-human/rat Yes Antibody

ORDERING INFORMATION

Catalog Number: AF3205

Lot Number: WGB01

Size: 100 µg (sufficient for 200 mL of blotting solution)

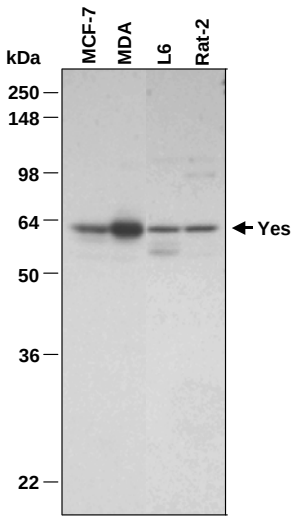
Storage: -20° C

Specificity: human and rat Yes

Immunogen: *E. coli*-derived rhYes (aa 1 - 88)

Ig Type: goat IgG

Applications: Western blot
Immunocytochemistry



Detection of Yes with AF3205. Lysates of human MCF-7, human MDA-MB-468, rat L6, and rat Rat-2 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 0.5 µg/mL anti-Yes, as described in *Protocols for Immunoblotting*. A twenty second exposure to film is shown.

Background

Yes is the cellular homolog of the oncogenic protein encoded by the Yamaguchi 73 and Esh avian sarcoma viruses. After Src, Yes is the most widely expressed member of the Src family of nonreceptor tyrosine kinases. Src family kinases regulate an array of cellular processes, including growth factor signaling, cytoskeleton dynamics, and cell proliferation.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human Yes protein (rhYes; aa 1 - 88; Accession # NM_005433). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human and rat Yes at 62 kDa using Western blot.

Applications

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Immunocytochemistry - This antibody can be used at a concentration of 10 µg/mL to detect Yes in ES cells. Cells were fixed with PBS containing 4% paraformaldehyde for 20 minutes at room temperature and blocked with PBS containing 10% normal donkey serum, 0.1% Triton X-100, and 1% BSA for 45 minutes at room temperature. After blocking, cells were incubated with diluted primary antibody overnight at 4° C followed by Rhodamine Red-coupled anti-goat IgG at room temperature in the dark for one hour. Between each step, cells were washed with PBS containing 0.1% BSA.

Optimal dilutions should be determined by the individual laboratory.

R&D Systems, Inc.
1-800-343-7475

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	5% nonfat dry milk in Blotting Buffer
0.15 M NaCl	Adjust pH to 7.4	Adjust pH to 7.4
0.1% Tween 20		

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.5 μg/mL anti-human/rat Yes.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo Chemiluminescent Detection Reagent (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5-10 seconds each.