

Biotinylated Anti-human/mouse/rat XIAP Antibody

ORDERING INFORMATION

Catalog Number: BAF8221

Lot Number: JQU01

Size: 50 µg

Storage: -20° C

Reconstitution: sterile 0.1% BSA in TBS

Specificity: human, mouse, and rat XIAP

Immunogen: *E. coli*-derived recombinant human XIAP

Ig Type: goat IgG

Application: Western blot

Background

XIAP (X-chromosome linked inhibitor of apoptosis), also known as MIHA, is a member of the inhibitor of apoptosis (IAP) family of proteins. XIAP directly interacts with caspases through its BIR domains to inhibit activity of caspase-3, -7 and -9.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human XIAP amino acids 1 - 497 fused with the amino acid sequence (MATVIDHHHHHSSNG) at the amino terminus (GenBank Accession # U45880). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein.

Formulation

Lyophilized from a 0.2 µm-filtered solution in phosphate-buffered saline (PBS) containing 50 µg of bovine serum albumin (BSA) per 1 µg of antibody.

Reconstitution

Reconstitute with sterile Tris-buffered saline pH 7.3 (20 mM Trizma base, 150 mM NaCl) containing 0.1% BSA. If 1 mL of buffer is used, the antibody concentration will be 50 µg/mL.

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

The antibody detects recombinant and endogenous human, mouse, and rat XIAP.

Applications

Western blot - An antibody concentration of 0.25 µg/mL is recommended.

Optimal dilutions should be determined by each laboratory for each application.

Protocols for Immunoblotting:

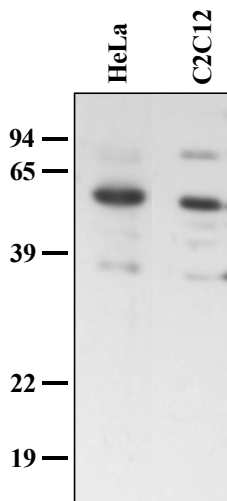
Western blotting

Blotting buffer	Blocking/Antibody Solution	HRP-Streptavidin Solution
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	2% BSA 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 an Immobilon filter (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 0.25 µg/mL goat anti-XIAP.
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 for 1 hour at room temperature in 2% BSA in blotting buffer containing a 1:200 - 1:2,000 dilution of HRP-conjugated Streptavidin (R&D Systems, Catalog # DY998).
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 2×10^6 - 1×10^7 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. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Tween is a registered trademark of ICI Americas.



Detection of XIAP with BAF8221.

Lysates from human HeLa and mouse C2C12 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.25 µg/mL biotinylated goat anti-XIAP, as described in *Protocols for Immunoblotting*. A one minute exposure to film is shown.