Affinity-Purified Sheep Anti-human BIK Antibody

ORDERING INFORMATION

Catalog Number: AF5474

Lot Number: CBPS01

Size: 100 µg

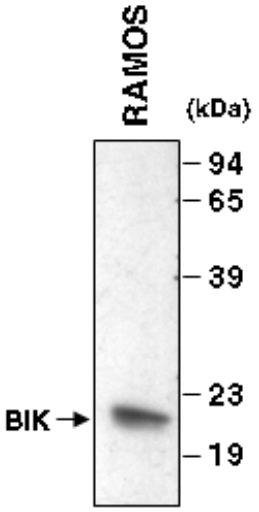
Storage: -20° C

Specificity: human BIK

Immunogen: *E. coli*-derived recombinant human BIK (aa 1 - 135)

Ig Type: sheep IgG

Applications: Western blot
Immunohistochemistry



Detection of BIK with AF5474.
Lysates from human Ramos cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 1.0 µg/mL sheep anti-BIK as described in *Protocols for Immunoblotting*.

Background

Bcl-2-interacting killer (BIK; also known as NBK, BP4 and BIP-1) is a 19 kDa member of the BH3-only subfamily, Bcl-2 family of proteins. It is widely expressed, and serves as an initiator of cell death. It apparently participates in apoptosis by sequestering antiapoptotic cytoplasmic proteins of the Bcl-2 family and in autophagy by regulating cytosolic calcium levels. Human BIK-1 is 160 amino acids (aa) in length. It contains one BH3 domain (aa 57 - 74), a "death segment" (aa 121 - 134) and a transmembrane domain (aa 136 - 156). BIK is likely to embed in both outer mitochondrial and ER membranes. Over amino acids 1 - 135, human BIK shares only 45% aa identity with mouse BIK.

Preparation

Produced in sheep immunized with purified, *E. coli*-derived, recombinant human Bcl-2 interacting killer (rhBIK; aa 1 - 135; Accession # Q13323). Human BIK specific IgG was purified by affinity chromatography.

Formulation

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

Reconstitution

Reconstitute the antibody in 100 µL PBS containing 0.02% NaN_3. The antibody concentration will be 1.0 mg/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 endogenous human BIK at ~22 kDa by Western blot.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane in antibody solution containing 1.0 µg/mL sheep anti-human BIK.
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 Antibody Solution containing a 1:2,000 dilution of HRP-conjugated donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detect with chemiluminescent reagents.

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 3 - 4 bursts of 5 - 10 second each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

Immunohistochemistry - This antibody will detect BIK in cells and tissues. The working dilution is 3 - 10 µg/mL. Antigen retrieval is recommended.

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

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