Affinity-purified Rabbit Anti-mouse BLK Antibody

ORDERING INFORMATION

Catalog Number: AF849

Lot Number: CNX02

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS

Storage: -20° C

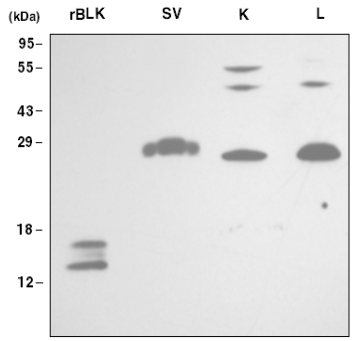
Reconstitution: sterile PBS

Specificity: mouse BLK

Immunogen: amino acids 25 - 42 of mouse BLK

Ig Type: rabbit IgG

Application: Western blot



Immunoblots of extracts of *E. coli* expressing amino acids 1 - 124 of recombinant mouse BLK (rBLK). Samples were electrophoresed on 15% gels and immunoblotting was with 1.0 µg/mL anti-BLK. Also shown are immunoblots of extracts from mouse seminal vesicles (SV), kidney (K), and liver (L). A 27 kDa polypeptide is detected in the tissue extracts by the anti-BLK. A one minute exposure is shown.

Preparation

Rabbits were immunized with the KLH coupled synthetic peptide VASETPSMKEPVR DVDLMC corresponding to amino acids 25 - 42 of mouse BLK a BH3-containing protein that interacts with Bcl-2 and Bcl-xL that is a potent death agonist. Cysteine was added to the carboxyl-terminal for coupling to KLH and for coupling to affinity matrix. Polyclonal antibody was affinity-purified on a column derivitized with the peptide.

Formulation

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

Reconstitution

Reconstitute in 100 µL of PBS containing 0.02% NaN_3.

Storage

Avoid repeated freezing and thawing by aliquoting smaller portions of the reconstituted antibody into Eppendorf tubes and storing at -20° C.

Specificity

The antibody detects mouse BLK.

Application

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

Protocols for Immunoblotting

Western blotting

Blotting buffer	Blocking solution	Antibody solution
25 mM Tris, pH 7.5	2% nonfat dry milk in blotting buffer	1% nonfat dry milk in blotting buffer
0.15 M NaCl	pH to 7.5	pH to 7.5
0.05% Tween 20		

1. Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in blocking solution.
2. Incubate the membrane overnight in antibody solution containing 1.0 µg/mL rabbit anti-mouse BLK.
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 in antibody solution containing a 1:2,000 dilution of HRP-conjugated Protein A (Amersham).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Visualize the immunodetected bands using the ECL system of Amersham.

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, 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. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Tissue extracts for western blotting: To prepare tissue extracts, tissue is excised, rinsed with cold PBS, minced and homogenized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and the extract is heated in a boiling water bath for 3 - 5 minutes. The extract is centrifuged at 12,000 x g to remove insoluble material.

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