Affinity-Purified Rabbit Anti-rat BOK

ORDERING INFORMATION

Catalog Number: AF825

Lot Number: BVU025121

Size: 80 µg

Storage: -20° C

Specificity: rat BOK

Immunogen: aa 41 - 61 of rat BOK

Ig Type: rabbit IgG

Applications: Western blot

Preparation
Rabbits were immunized with the KLH coupled, synthetic peptide, ARLLRAGLSWSAPERASPAPGC (corresponding to amino acids 41 - 61 of rat BOK). Cysteine was added to the carboxyl-terminal for coupling to KLH and for coupling to an affinity matrix. Polyclonal antibody was affinity-purified on a column derivatized with the peptide.

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

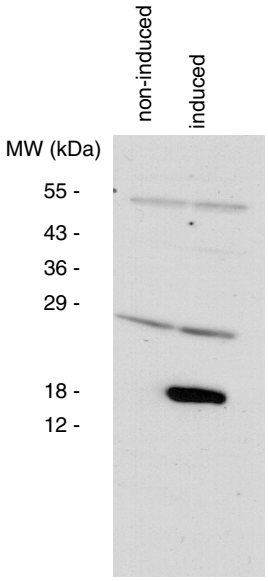
Reconstitution
Reconstitute the antibody in 100 µL of PBS containing 0.02% NaN_3.

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 recombinant rat BOK expressed in *E. coli* cells.

Western blot
An antibody concentration of 0.8 µg/mL is recommended. The ability of the antibody to blot endogenous BOK in cell extracts is not known.

Immunoblots of SDS-extracts from *E. coli* transfected with an inducible expression vector containing rat BOK. Immunoblots of SDS extracts from non-induced and induced cells are shown. Extracts were electrophoresed on 15% gels and immunoblots were with 0.8 µg/mL anti-rat BOK. Incubation with anti-BOK was overnight at 4° C and detection was by the ECL procedure (Amersham). A 15 second exposure is shown.



15 second exposure

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

Protocols for Immunoblotting with Affinity-purified Rabbit Anti-rat BOK

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 at 4° C in antibody solution containing 0.8 μg/mL rabbit anti-BOK.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer. Changing 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 Protein A (Amersham).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detection was with ECL Reagent (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 2×10^6 - 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.