



## 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<sub>3</sub>.

### Storage

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

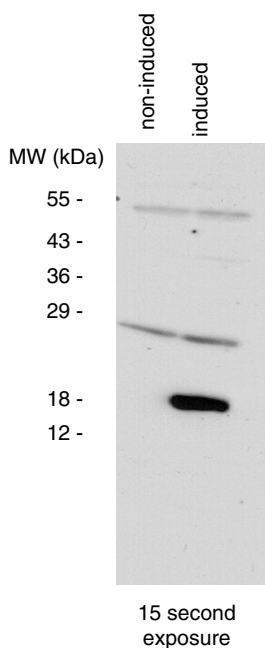
### Specificity

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.



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

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

### **Western blotting**

<u>Blotting buffer</u>	<u>Blocking solution</u>	<u>Antibody solution</u>
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  $2 \times 10^6$  -  $1 \times 10^7$  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.