NEUROMICS

Bad Data Sheet

Catalog Number:	RA15050	**Host:**	Goat
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human
Immunogen Sequence:	KLH coupled synthetic peptide, MFQIPEFEPSEQEDSSSAERGC (corresponding to amino acids 1 - 21 of human Bad). Cysteine was added to the carboxyl-terminal for coupling to an affinity matrix.	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose

Applications: Western Blot-1 µg/mL

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

Application Notes

Specificity

The antibody is known to react with human and mouse Bad.

Western Blot

An antibody concentration of 1.0 µg/mL is recommended.

Western Blot Protocol

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.5 0.15 M NaCl 0.05% Tween 20	5% nonfat dry milk in blotting buffer pH 7.5	2% nonfat dry milk in blotting buffer pH 7.5

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 buffer containing 1.0 µg/mL rabbit anti-Bad.

3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer.

4. Incubate the membrane for 1 hour at room temperature in antibody buffer 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).

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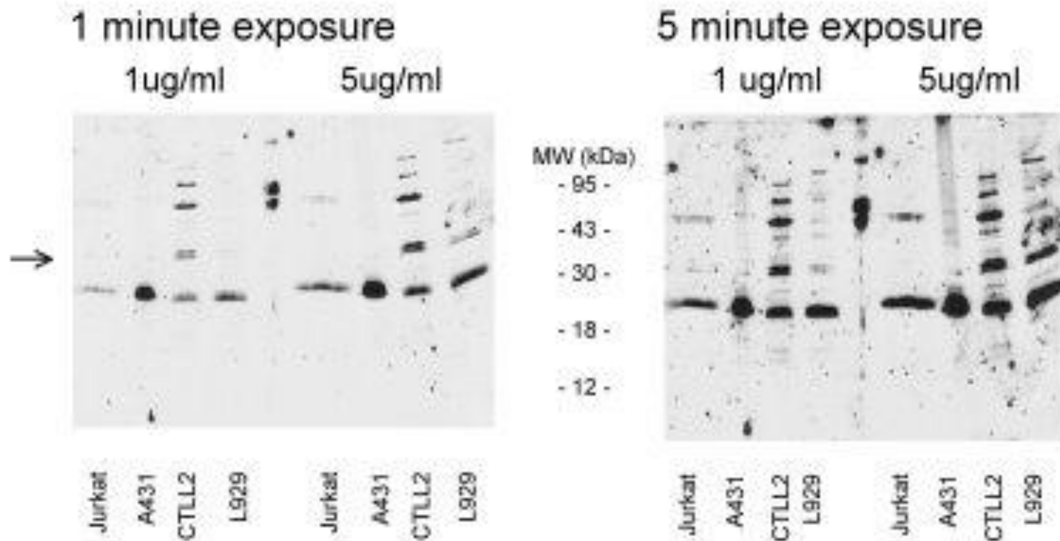
Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at 2 x 106 - 1 x 107 cells per mL. The extracts are heated in a boiling water bath for 5 minutes 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.



Immunoblots of SDS-extracts from human Jurkat (3 x 105), A431 (2 x 105), mouse CTLL2 (2.5 x 105), and L929 (2 x 105) cells. Extracts were blotted with 1.0 and 2.0 µg/mL anti-Bad. Incubation with anti-Bad was overnight at 4° C and detection was by the ECL procedure (Amersham). One and five minute exposures are shown. The band that co-migrates with recombinant Bad is indicated by the arrow. The identity of the other bands is unknown.

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