upstate | CHEMICON | Linco

THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

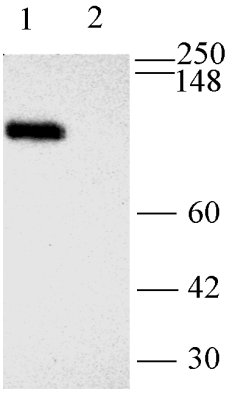
# RABBIT ANTI-POTASSIUM CHANNEL MAXI K ($BK_{Ca}$) AFFINITY PURIFIED POLYCLONAL ANTIBODY

| | | | |
|---|---|---|---|
| **CATALOG NUMBER:** | AB5228-50UL | **QUANTITY:** | 200 µL |
| **LOT NUMBER:** | | **CONCENTRATION:** | 0.4 mg/mL (after reconstitution) |

**SPECIFICITY:** Recognizes the C-terminus of the longer form of $BK_{Ca}$ channel α subunit and overlaps by ~40 residues with a shorter form and not with other known proteins tested so far.

**IMMUNOGEN:** A purified fusion protein (GST) and a C-terminal part (residues 1097-1196) of mouse $K_{Ca}$ 1.1 channel (Accession Q08460-2)

**APPLICATIONS:** Western blot: 1:200 using ECL on rat brain membranes.
Note: The addition of 0.1% Tween to the standard milk block is recommended.
Immunohistochemistry on rat brain sections.
Dilutions should be made using a carrier protein such as BSA (1-3%)
Optimal working dilutions must be determined by the end user.




Western blotting of rat brain membranes (25 µg/lane)
AB5228 (1:200)
AB5228 preincubated with control peptide

**SPECIES REACTIVITY:** Rat. Other species have not been tested.

**CONTROL:** Included free of charge with the antibody is 120 µg of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 µL of PBS. For positive control, in Western blotting, use 20 ng protein per minigel lane. For negative control, preincubate 3 µg of purified peptide with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.

**FORMAT:** Affinity purified immunoglobulin.

EMD Millipore Corporation, 28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com

**PRESENTATION:** Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA and 0.025% sodium azide as a preservative. Reconstitute with 200 µL of sterile deionized water. Centrifuge antibody preparation before use (10,000 xg for 5 min).

**STORAGE/HANDLING:** Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

**REFERENCES:**

1. Xu, H *et al.* (2005) *Hypertension* 46, 1154
2. Muinuddin, A *et al* (2005) *Am. J. Physiol.* 288, G1233
***3.*** Werner, M.E. *et al. (2005) J. Physiol.* 567.2, 545

## SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples (organ membranes: 50 µg/lane; transfected cells: 500,000 cells/lane) with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 µL applied to Minigel lane (0.75-1.5 mm width) and run at standard conditions. (60 mA for 2 1.5 mm Minigel gels, 1.4 h). It is suggested that you run 5-15% acrylamide (37.5:1 acrylamide:bisacrysmide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
3. Transfer in semi-dry system under standard conditions (3 h 100 mA for two minigel gels)
4. Stain the transferred bands with BLOT-*FastStain* (Catalog Number 2076).
5. Destain with deionized water.
6. Block with 5% non-fat milk (Marvel or Carnation) in PBS, and 0.025 % sodium azide, overnight at 2-8°C. The non-fat milk should be dissolved freshly, centrifuged 10,000 rpm for 10 min, and filtered through glass filter (Gelman Acrodisc).
7. Incubation with first antibody 2 h at room temperature or overnight at 4°C in blocking solution. The antibody preparation should be centrifuged before use (10,000 g 5 min.). Optimal working dilutions and incubation time will need to be determined by the end user.
8. Wash 4 x 10 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
9. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
10. Wash 4 x 10 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (Chemilucent, Catalog Number 2600).

***Important Note:*** *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION