upstate | CHEMICON | Linco

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

RABBIT ANTI-K_{Ca}2.2 (SK2) AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5356-50UL

LOT NUMBER:

QUANTITY: 50 µL

CONCENTRATION: 0.8 mg/mL (after reconstitution)

SPECIFICITY: Recognizes K_{Ca}2.2 (SK2, KCa2, Kcnn2, SKCa2, Apamin-Sensitive Small Conductance Ca^{2+}-dependent K^+ Channel). The epitope specific for K_{Ca}2.2 is not present in any other known proteins.

IMMUNOGEN: Highly purified peptide corresponding to residues 542-559 of rat K_{Ca}2.2 (Accession P70604).

APPLICATIONS: Western blotting: 1:200 using ECL on rat brain membranes.
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.

CONTROL ANTIGEN: Included free of charge with the antibody is XX µg of control antigen (lyophilized powder). For negative control, preincubate 1 µg of purified peptide with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.

SPECIES REACTIVITIES: Rat. Reactivity with other species has not yet been tested. The immunogen sequence is conserved in mouse (17/18), human (16/18) and chicken (14/18).

FORMAT: Affinity purified immunoglobulin.

PRESENTATION: Lyophilized from PBS, pH 7.4, containing 1% BSA, and 0.025% sodium azide. Reconstitute with 50 µL of sterile distilled water. Centrifuge antibody preparation before use (10,000 x g for 5 min).

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

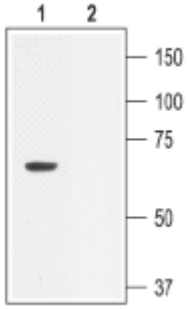
28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com

upstate | CHEMICON | Linco

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

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 Chemicon 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 Chemicon 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, Chemicon Catalog Number 2600).



Western blotting of rat brain membranes:
1. AB5356, 1:200
2. AB5356, preincubated with the control peptide

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

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

upstate | CHEMICON | Linco

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

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