

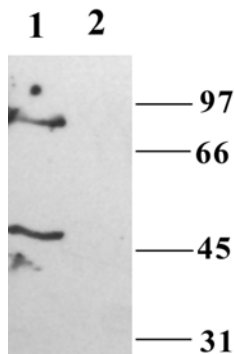


**RABBIT ANTI-TWIK-2
(Kcnk6, TOSS)
AFFINITY PURIFIED, POLYCLONAL ANTIBODY**

CATALOG NUMBER:	AB5572-200UL
LOT NUMBER:	
QUANTITY:	200 μ L
CONCENTRATION:	120 μ g/200 μ L (after reconstitution)
SPECIFICITY:	Recognizes TWIK-2. Does not cross react with any other known proteins.
IMMUNOGEN:	Peptide corresponding to amino acids 295-313 from human TWIK-2 (1,2).
APPLICATIONS:	Western blot: 3.0 μ g/mL (1:200) using ECL on rat brain membranes. 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 μ g of control antigen. The stock solution of the antigen can be made up using 100 μ L of sterile deionized water. For negative control, preincubate 1 μ g of peptide with 1 μ g of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.
SPECIES REACTIVITIES:	Rat. Other species have not been tested.
FORMAT:	Affinity purified immunoglobulin.
PRESENTATION:	Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA, 5% sucrose as a stabilizer and 0.025% sodium azide as a preservative. Reconstitute with 200 μ L 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 reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.
REFERENCES:	<ol style="list-style-type: none">1. Chavez, R.A., <i>et al.</i> (1999) <i>J. Biol. Chem.</i> 274, 7887.2. Pountney, D.J., <i>et al.</i> (1999) <i>FEBS Lett.</i> 450, 191.

SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 μ L 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)
3. Transfer in semi-dry system under standard conditions (3 h 200 mA for two minigel gels)
4. Stain the transferred bands with Millipore 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 4°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 15 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 Millipore Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
10. Wash 4 x 15 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (*femto*LUCENT, Millipore Catalog Number 2078).



Western blotting of rat brain membranes (50 μ g)

1. AB5572 antibody (1:200).
2. AB5572 antibody, preincubated with the control peptide antigen.

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 μ L 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.*

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