

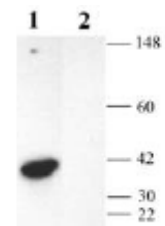
RABBIT ANTI-SYNTAXIN 4 AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER:	AB5330-50UL	QUANTITY:	50 μ L
LOT NUMBER:		CONCENTRATION:	0.4 mg/mL (after reconstitution)
SPECIFICITY:	Recognizes Syntaxin 4.		
IMMUNOGEN:	Highly purified corresponding to residues 2-23 of rat or mouse Syntaxin 4 (Accession Q08850).		
APPLICATIONS:	Western blotting: 1:200-1:500 using ECL on rat kidney and heart membranes.		

Western blotting of rat kidney membranes:

Lane 1: AB5330, 1:600

Lane 2: AB5330, preincubated with the control peptide antigen.



Dilutions should be made using a carrier protein such as BSA (1-3%).
Optimal working dilutions must be determined by the end user.

SPECIES REACTIVITY:	Reacts with Rat. Reactivity with other species has not been determined. The immunogen sequence is identical in mouse and 17/22 amino acids identical in human.
CONTROL:	Included free of charge with the antibody is XX μ g of control antigen (lyophilized powder). Reconstitute with 100 μ L of sterile distilled water. For negative control, preincubate 1 μ g of purified peptide with 1 μ g of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.
FORMAT:	Affinity purified immunoglobulin.
PRESENTATION:	Lyophilized from PBS, pH 7.4, containing 1% BSA and 0.025% sodium azide. Reconstitute with 50 μ L 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 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:	Pombo, L. et al. (2001) <i>J. Biol. Chem.</i> 276, 42893 Rao, S.K et al. (2004) <i>J. Biol. Chem.</i> 279, 20471



SUGGESTED WESTERN BLOT PROTOCOL

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.

1. 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:bisacrylamide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
2. Transfer in semi-dry system under standard conditions (3 h 100 mA for two minigel gels)
3. Stain the transferred bands with Chemicon BLOT-*FastStain* (Catalog Number 2076).
4. Destain with deionized water.
5. 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).
6. 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.
7. Wash 4 x 10 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
8. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Chemicon Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
9. Wash 4 x 10 min. with PBS-0.1% tween 20.
10. Perform ECL with commercial kits (Chemiluminescent, Chemicon 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

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