

Anti-phospho-beta 2-Adrenergic Receptor (Thr384)

(rabbit polyclonal IgG)

Catalog # 07-629

Lot # 27080

Immunogen: KLH-conjugated, synthetic peptide containing the sequence ...PGpTED... in which pT corresponds to phosphothreonine at residue 384 of human beta 2-Adrenergic Receptor. The immunizing sequence is identical in monkey.

Specificity: Recognizes phospho-beta 2-Adrenergic Receptor (Thr384), Mr 43kDa.

Species Cross-reactivity: Human, mouse, and rat. Predicted to cross-react with monkey based on sequence homology.

Formulation: 200µg of protein A purified rabbit IgG in 244µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Ligated at -20°C.

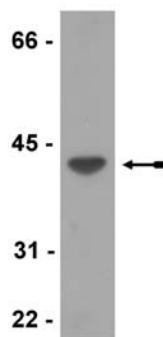
Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 0.2-2µg/ml of this lot detected phospho-beta 2-Adrenergic Receptor (Thr384) in RIPA lysates from 3T3/A31 cells.

Included Positive Antigen Control: Catalog # 12-305, 3T3/A31 cell lysate. Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation. Load 20µg of reduced lysate per lane for minigels.



Immunoblot Analysis

3T3/A31 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-beta 2-Adrenergic Receptor (Thr384) (0.5µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phospho-beta 2-Adrenergic Receptor (Thr384) (~43kDa).

General References:

1. Seibold, A., *et al.*, *J. Biol. Chem.* **273**, 7637-7642, 1998.
2. Tran, T.M., *et al.*, *Mol. Pharmacol.* **65**, 196-206, 2004.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄, 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 5% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween[®]-20 (TBST-MLK) for 2 hours at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.2-2 μ g/ml of anti-phospho-beta 2-Adrenergic Receptor (Thr384)**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 2 hours with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween[®]-20 for 15 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Wash the nitrocellulose in water at room temperature for 1 hour with agitation.
10. Use detection method of choice (enhanced chemiluminescence was used).