Affinity-Purified Rabbit Anti- Phospho-Progesterone R/NR3C3 (S294) Antibody

ORDERING INFORMATION

Catalog Number: AF5955

Lot Number: CDLG01

Size: 100 µg

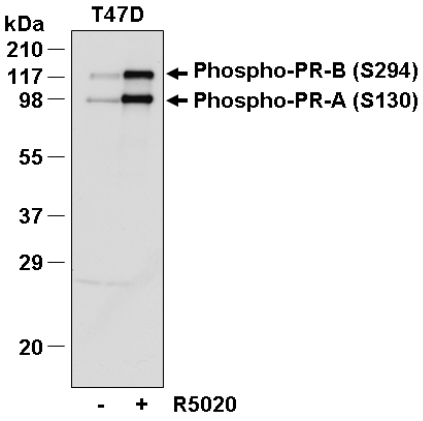
Storage: -20° C

Specificity: human Phospho-PR (S294)

Immunogen: phosphopeptide containing human PR-B S294 site

Ig Type: rabbit IgG

Application: Western blot



Detection of phosphorylated PR-A and PR-B with AF5955. Human T47D cells were either untreated or treated for 60 minutes with 100 nM of the synthetic progestin promegestone (R5020). Total cell lysates in gel sample buffer were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 0.5 µg/mL rabbit anti-Phospho-PR (S294), as described in *Protocols for Immunoblotting*.

Background

Progesterone Receptor B (PR-B) is a 118 kDa member of the NR3 subfamily within the nuclear hormone receptor family of proteins. It is expressed in female reproductive tissues as well as neurons throughout the CNS. PR-B is particularly important in the mammary gland where it mediates proliferative responses to progesterone. Human PR-B is 933 amino acids (aa) in length. It contains an N-terminal regulatory region (aa 1 - 566), a DNA binding domain (aa 567 - 639), and a steroid-binding region (aa 681 - 933). Ligand binding induces a key phosphorylation event at Ser294 catalyzed by ERK1/2, regulating cell growth, cell adhesion, and gene transcription. An alternate start site at Met165 generates 90 kDa PR-A, an isoform particularly important in the ovary and uterus that insures fertility.

Preparation

Rabbit antibodies were raised against a synthetic phosphopeptide corresponding to residues surrounding S294 of human PR-B (Accession # P06401). Polyclonal antibody was affinity-purified on a column derivatized with the phosphopeptide, and further purified by protein A chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects endogenous human PR-B and PR-A when phosphorylated at S294 (118 kDa) and S130 (90 kDa), respectively, using Western blots.

Application

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Antibody Solution containing 0.5 µg/mL rabbit anti-Phospho-PR (S294).
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-rabbit IgG (R&D Systems, Catalog # HAF008).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL.

Optimal dilutions should be determined by each laboratory for each application.

R&D Systems, Inc.
1-800-343-7475

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