Monoclonal Anti-Phospho-PR (S294)
Certificate of Analysis

ORDERING INFORMATION

Catalog Number: PPS018

Clone: 608

Lot Number: 1489083

Size: 100 µL (sufficient for 10 mini blots)

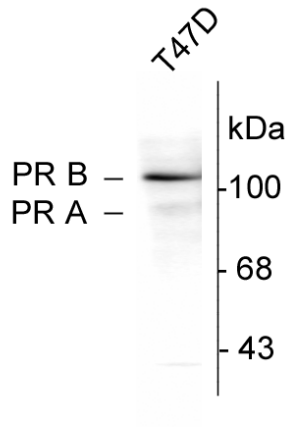
Storage: ≤ -20° C

Specificity: Human ~90 kDa PR-A isoform and the ~120 kDa PR-B isoform phosphorylated at S294

Immunogen: Phosphopeptide corresponding to amino acid residues surrounding the phospho-S294 of human Progesterone Receptor.

Ig Type: mouse IgG_1

Applications: Western blot
Immunohistochemistry



Western blot of whole cell T47D lysate prepared from cells that had been incubated in the presence of the synthetic progestin agonist R5020 (500 nm) showing specific labeling of the ~90 kDa PR-A isoform and the ~120 kDa PR-B isoform of the Progesterone Receptor phosphorylated at S294. The labeling by the antibody was specifically blocked by the phosphopeptide used as antigen. The corresponding dephosphopeptide did not block the immunolabeling (not shown).

Description
In humans, the progesterone receptor (PR) gene gives rise to multiple isoforms. The “B” (PR -B), or long form, is 116 kDa in size and 933 amino acids (aa) in length. It contains a proline-rich N-terminal region (aa 1 - 566), a central DNA-binding domain (DBD) (aa 567 - 636), a nuclear localization motif (aa 637 - 644), and a hormone binding/dimerization domain (HBD) (aa 645 - 933). PR-A utilizes a different start site that shortens the N-terminus by 164 amino acids. It is 94 kDa in size and 769 aa in length. The N-terminus in both is rich in serine that is phosphorylated in response to hormone binding. In the absence of hormone, a few PR-A and -B molecules are phosphorylated at Serine 190. Hormone increases this number two-fold, providing evidence for hormone stimulation. The common Serine at 294 can only be phosphorylated on PR-B, due to a difference in N-terminal conformation. This may account for functional differences between the molecules. Alternate start sites also generate two shorter forms that lack the N-terminus. PR-C is 60 kDa in size and 339 aa in length; PR-M is 38 kDa in size and 314 aa in length. PR-A, -B and -C are known to heterodimerize. Alternate splicing of PR-A generates at least four other isoforms. All contain aa 1 - 516 (with the N-terminus), and are either truncated or show a partial deletion of the HBD.

Preparation
Prepared by affinity purification using a Protein G column.

Formulation
100 µL in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/mL BSA and 50% glycerol.

Storage
For long-term storage, ≤ -20° C is recommended. Product is stable at ≤ -20° C for at least 1 year.

Specificity
Specific for the ~90 kDa PR-A isoform and the ~120 kDa PR-B isoform phosphorylated at S294 in Western blots of human brain extracts.

Applications
Western blot - 1:1000
Immunohistochemistry - 1:1000

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

References

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2. Wei, L.L. *et al*. (1996) Mol. Endocrinol. **10**:1379.
3. Saner, K.J. *et al*. (2003) Mol. Cell. Endocrinol. **200**:155.
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5. Richer, J.K. *et al*. (1998) Breast Cancer Res. Treat. **48**:231.
6. Knotts, T.A. *et al*. (2001) J. Biol. Chem. **276**:8475.
7. Clemm, D.L. *et al*. (2000) Mol. Endocrinol. **14**:52.

H. Helen Zhang

Director, Quality Assurance

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