

Progesterone Receptor Signaling Antibody Sampler Kit

✓ 1 Kit
 (6 x 40 µl)



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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	4370	40 µl	44, 42 kDa	Rabbit IgG
Phospho-Progesterone Receptor (Ser190) Antibody	3171	40 µl	90, 118 kDa	Rabbit IgG
Phospho-Progesterone Receptor (Ser345) Antibody	12783	40 µl	90, 118 kDa	Rabbit IgG
Progesterone Receptor A/B (D8Q2J) XP® Rabbit mAb	8757	40 µl	90, 118 kDa	Rabbit IgG
Progesterone Receptor B (C1A2) Rabbit mAb	3157	40 µl	118 kDa	Rabbit IgG
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	40 µl	60 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: Progesterone Receptor Signaling Antibody Sampler Kit provides an economical means of detecting total and active levels of progesterone receptor (PR) as well as the active forms of PR downstream targets. The kit contains enough primary antibody to perform four western blots per primary antibody.

Background: Human progesterone receptor (PR) is expressed as two forms: the full length PR-B and the short form PR-A. PR-A lacks the first 164 amino acid residues of PR-B (1,2). Both PR-A and PR-B are ligand activated, but differ in their relative ability to activate target gene transcription (3,4). The activity of PR is regulated by phosphorylation; at least seven serine residues are phosphorylated in its amino-terminal domain. Three sites (Ser81, Ser102, and Ser162) are unique to full length PR-B, while other sites (Ser190, Ser294, Ser345, and Ser400) are shared by both isoforms (5). Phosphorylation of PR-B at Ser190 (equivalent to Ser26 of PR-A) is catalyzed by CDK2 (6). Mutation of Ser190 results in decreased activity of PR (7), suggesting that the phosphorylation at Ser190 may be critical to its biological function. Research studies have demonstrated ligand-dependent phosphorylation of PR-B at Ser345 is catalyzed by MAPK and plays an important role in mediating the proliferation of breast cancer cells. Investigators have shown that Ser345-phosphorylated PR-B associates with Sp1 to regulate EGFR and p21 transcription (8). PR signaling has been shown to crosstalk with other kinase signaling cascades. Upon stimulation and the subsequent interaction with estrogen receptor α and c-Src, PR-B has been shown to promote the activation of the Src/p21^{ras}/Erk pathway (9).

Specificity/Sensitivity: Unless otherwise indicated, each antibody will recognize endogenous total levels of target protein. Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb recognizes endogenous p44 and p42 MAP Kinase (Erk1/2) when dually phosphorylated at Thr202/Tyr204 of Erk1 (Thr185/Tyr187 of Erk2), and singly phosphorylated at Thr202. This antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP kinases. Phospho-Progesterone Receptor (Ser190) Antibody detects endogenous levels of progesterone receptors B and A only when phosphorylated at Ser190 and Ser26, respectively. Phospho-Progesterone Receptor (Ser345) Antibody recognizes endogenous levels of progesterone receptors B and A only when phosphorylated at Ser345 and Ser181, respectively. Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb may cross-react with other Src family proteins phosphorylated at equivalent sites or with overexpressed phosphorylated RTKs.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase, or with synthetic peptides corresponding to residues surrounding Ser115 or Tyr541 of human progesterone receptor protein, or Tyr416 of human Src protein. Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser190 of human progesterone receptor protein or Ser345 of human progesterone receptor B protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
 Western blotting 1:1000

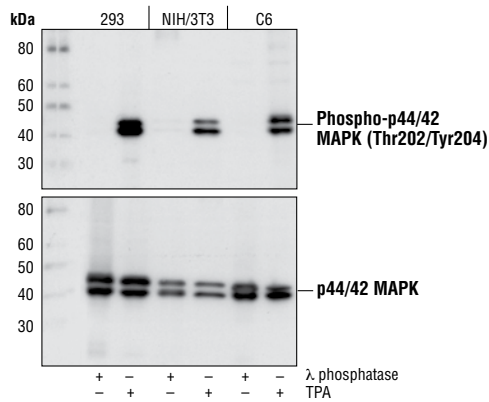
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

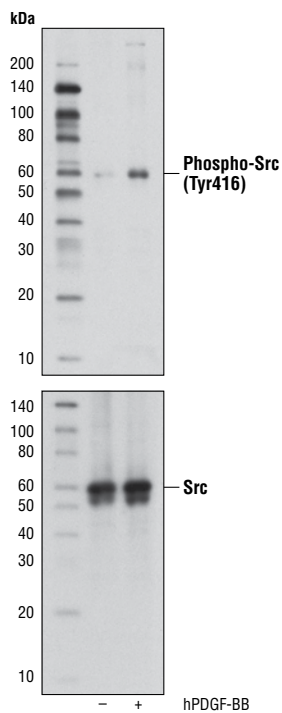
- (1) Evans, R.M. (1988) *Science* 240, 889-95.
- (2) Kastner, P. et al. (1990) *EMBO J* 9, 1603-14.
- (3) Giangrande, P.H. et al. (2000) *Mol Cell Biol* 20, 3102-15.
- (4) Wen, D.X. et al. (1994) *Mol Cell Biol* 14, 8356-64.
- (5) Clemm, D.L. et al. (2000) *Mol Endocrinol* 14, 52-65.
- (6) Zhang, Y. et al. (1997) *Mol Endocrinol* 11, 823-32.
- (7) Takimoto, G.S. et al. (1996) *J Biol Chem* 271, 13308-16.
- (8) Faivre, E.J. et al. (2008) *Mol Endocrinol* 22, 823-37.
- (9) Blunt, R.J. et al. (1975) *Pflügers Arch* 355, 189-204.

U.S. Patent No. 5,675,063

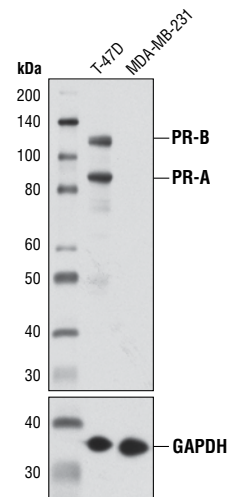
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



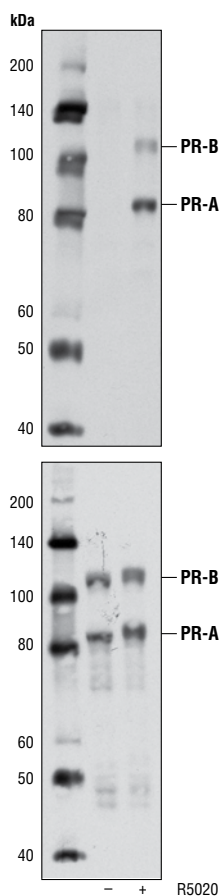
Western blot analysis of extracts from 293, NIH/3T3 and C6 cells, treated with λ phosphatase or TPA #4174 as indicated, using **Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP[®] Rabbit mAb #4370** (upper), or p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).



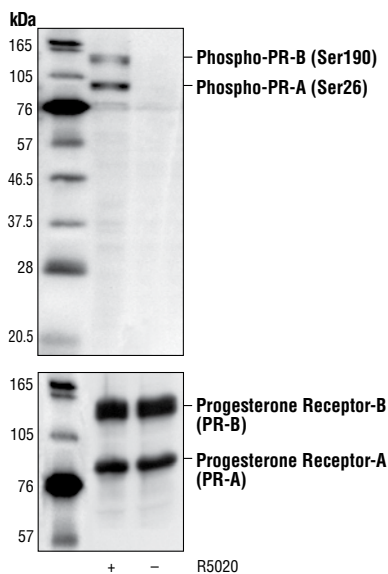
Western blot analysis of extracts NIH/3T3 cells, serum-starved (-) or treated with human Platelet-Derived Growth Factor BB hPDGF-BB #8912 (100 ng/ml, 15 min; +), using **Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb #6943** (upper) or Src (36D10) Rabbit mAb #2109 (lower).



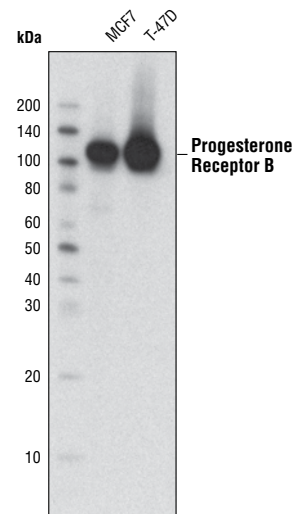
Western blot analysis of extracts from T-47D (PR positive) and MDA-MB-231 (PR negative) cells using **Progesterone Receptor A/B (D8Q2J) XP[®] Rabbit mAb #8757** (upper) or GAPDH (D16H11) XP[®] Rabbit mAb #5174 (lower).



Western blot analysis of extracts from T-47D cells grown for 48 hr in phenol red-free medium supplemented with 5% charcoal-stripped FBS, untreated (-) or promegestone (R5020)-treated (100 nM, 1 hr; +), using **Phospho-Progesterone Receptor (Ser345) Antibody #12783** (upper) or Progesterone Receptor A/B (D8Q2J) XP[®] Rabbit mAb #8757 (lower).



Western blot analysis of extracts from T-47D cells, untreated (-) or promegestone (R5020)-treated (100 nM, 1 hr; +), using **Phospho-Progesterone Receptor (Ser190) Antibody #3171** (upper) and control Progesterone Receptor Antibody #3172 (lower).



Western blot analysis of extracts from MCF7 and T-47D cells using **Progesterone Receptor B (C1A2) Rabbit mAb #3157**.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.
NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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