**Nuclear Receptor Antibody Sampler Kit**

- **1 Kit**  
  (8 x 20 µl)

**Products Included**

<table>
<thead>
<tr>
<th>Product</th>
<th>Product #</th>
<th>Quantity</th>
<th>Mol. Wt.</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen Receptor (D6F11) XP® Rabbit mAb</td>
<td>5153</td>
<td>20 µl</td>
<td>110 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Estrogen receptor α (D8H6) Rabbit mAb</td>
<td>8644</td>
<td>20 µl</td>
<td>66 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Glucocorticoid Receptor (D8H2) XP® Rabbit mAb</td>
<td>3660</td>
<td>20 µl</td>
<td>80, 91, 94 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>PPARγ (C26H12) Rabbit mAb</td>
<td>2435</td>
<td>20 µl</td>
<td>53, 57 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Progesterone Receptor A/B (D8O2J) XP® Rabbit mAb</td>
<td>8757</td>
<td>20 µl</td>
<td>90 (PR-A), 118 (PR-B) kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>RARα Antibody</td>
<td>2554</td>
<td>20 µl</td>
<td>55 kDa</td>
<td></td>
</tr>
<tr>
<td>RARγ1 (D3A4) XP® Rabbit mAb</td>
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<td>20 µl</td>
<td>58 kDa</td>
<td>Rabbit IgG</td>
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<tr>
<td>RXRα (D6H10) Rabbit mAb</td>
<td>3085</td>
<td>20 µl</td>
<td>53 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Anti-rabbit IgG, HRP-linked Antibody</td>
<td>7074</td>
<td>100 µl</td>
<td></td>
<td>Goat</td>
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</tbody>
</table>

**Description:** The Nuclear Receptor Antibody Sampler Kit provides an economical means to evaluate the presence and status of nuclear receptors. This kit includes enough antibody to perform two western blots with each primary antibody.

**Background:** Nuclear receptors are transcription factors responsible for sensing bioactive molecules, including steroid and thyroid hormones. They are regulated by multiple posttranslational modifications, which in turn impact their ability to regulate the expression of specific genes involved in the control of reproduction, development, and metabolism.

Androgen receptor (AR), a zinc finger transcription factor belonging to the nuclear receptor superfamily, is activated by phosphorylation and dimerization upon ligand binding (1). This promotes nuclear localization and binding of AR to androgen response elements in androgen target genes. AR plays a crucial role in several stages of male development and the progression of prostate cancer (2,3).

Estrogen receptor α (ERα), a member of the steroid receptor superfamily, contains highly conserved DNA binding and ligand binding domains (4). Through its estrogen-independent and estrogen-dependent activation domains (AF-1 and AF-2, respectively), ERα regulates transcription by recruiting coactivator proteins and interacting with general transcriptional machinery (5).

Glucocorticoid hormones control cellular proliferation, inflammation, and metabolism through their association with the glucocorticoid receptor (GR)/NR3C1, a member of the nuclear hormone receptor superfamily of transcription factors (6).

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the ligand-activated nuclear receptor superfamily and functions as a transcriptional activator (7). PPARγ is preferentially expressed in adipocytes, as well as in vascular smooth muscle cells and macrophages (8). Besides its role in mediating adipogenesis and lipid metabolism (8), PPARγ also modulates insulin sensitivity, cell proliferation, and inflammation (9).

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 (10,11). Both PR A and PR B are ligand activated, but differ in their relative ability to activate target gene transcription (12,13).

Nuclear retinoic acid receptors (RARs) consist of three subtypes encoded by separate genes: α (NR1A1), β (NR1B1), and γ (NR1B2), and γ (NR1B3). For each subtype, there are at least two isoforms, which are generated by differential promoter usage and alternative splicing and differ only in their N-terminal regions. Retinoids, which are metabolites of vitamin A, serve as ligands for RARs (14). RARs function as ligand-dependent transcriptional regulators and are found to be heterodimerized with retinoid X receptors (RXRs). These transcriptionally activedimers regulate the expression of genes involved in cellular differentiation, proliferation, and apoptosis (15,16).

The human retinoid X receptors are encoded by three distinct genes (RXRα, RXRβ, and RXRγ) and bind selectively and with high affinity to the vitamin A derivative, 9-cis-retinoic acid. RXRs are type-II nuclear hormone receptors that are largely localized to the nuclear compartment independent of ligand binding. Nuclear RXRs form heterodimers with nuclear hormone receptor subfamily 1 proteins, including thyroid hormone receptor, retinoic acid receptors, vitamin D receptor, peroxisome proliferator-activated receptors, liver X receptors, and farnesoid X receptor (17).

**Specificity/Sensitivity:** Each antibody in the Nuclear Receptor Antibody Sampler Kit recognizes endogenous levels of total respective protein. Glucocorticoid Receptor (D8H2) XP® Rabbit mAb is predicted to cross-react with all known alternative translation start site generated isoforms of glucocorticoid receptor-α and glucocorticoid receptor-β, and does not cross-react with mineralocorticoid receptor. Progesterone Receptor A/B (D8O2J) XP® Rabbit mAb does not cross-react with either the glucocorticoid receptor or the mineralocorticoid receptor. RARγ1 (D3A4) XP® Rabbit mAb is not predicted to cross-react with RARγ2, and does not cross-react with either RARα or RARβ. RXRα (D6H10) Rabbit mAb does not cross-react with either RXRβ or RXRγ.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence of human RARα protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminal region of human androgen receptor protein, residues in the carboxy terminus of human ERα protein, residues surrounding Leu378 of human glucocorticoid receptor protein, residues surrounding Asp69 of human PPARγ protein, residues surrounding Tyr541 of human progesterone receptor protein, residues near the amino terminus of human RARγ protein, or residues near the amino terminus of human RXRα protein.

**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 validation data and a complete listing of recommended companion products.
Background References:

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. 1X SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. 10X Tris Buffered Saline (TBS): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. Blocking Buffer: 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. Wash Buffer: 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween®20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
10. Phototope®-HRP Western Blot Detection System #7071: Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading precasted molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.