Phospho-Estrogen Receptor α Antibody Sampler Kit

1 Kit (4 x 20 µl)

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

**Description:** The Phospho-Estrogen Receptor α Antibody Sampler Kit provides an economical means to evaluate the activation status of ERα, including phosphorylation of Ser104/106 and Ser118. Phospho-Estrogen Receptor α (Ser167) Antibody detects endogenous levels of Ser167 phosphorylated ERα. Each antibody in the kit does not cross-react with phosphorylated or nonphosphorylated ERα isoforms β or other related family members.

**Background References:**

**Recommended Antibody Dilutions:**
- Western blotting: 1:1000

**Background:** Estrogen receptorα (ERα), a member of the steroid receptor superfamily, contains highly conserved DNA binding (DBD) and ligand binding domains (LBD) (1). 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 (2). Phosphorylation provides an important mechanism to regulate ERα activity (3,4). ERα is phosphorylated on multiple sites (5). Serines 104, 106, 118 and 167 are located in the amino-terminal transcription activation function domain AF-1, and phosphorylation of these serines plays an important role in regulating ERα activity. ERα activity. Ser118 may be the substrate of the transcription regulatory kinase cdK7 (5). Ser167 may be phosphorylated by p90RSK and Akt (4,6). Phosphorylation of Ser167 may confer tamoxifen resistance in breast cancer patients (4).

**Specificity/Sensitivity:** Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb detects endogenous levels of ERα only when phosphorylated at Ser167, and also cross reacts with a nonspecific band around 77 kDa. Phospho-Estrogen Receptor α (Ser104/106) Antibody detects endogenous levels of Ser104/106 phosphorylated ERα. Phospho-Estrogen Receptor α (Ser118) (16J4) Mouse mAb will detect endogenous levels of Ser118 phosphorylated ERα. Estrogen Receptor α (D8H8) Rabbit mAb detects late ERα activity (3,4). ERα is phosphorylated on multiple sites (5). Serines 104, 106, 118 and 167 are located in the amino-terminal transcription activation function domain AF-1, and phosphorylation of these serines plays an important role in regulating ERα activity. Ser118 may be the substrate of the transcription regulatory kinase cdK7 (5). Ser167 may be phosphorylated by p90RSK and Akt (4,6). Phosphorylation of Ser167 may confer tamoxifen resistance in breast cancer patients (4).

**Storage:** Supplied in 1 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C.

**Monoclonal antibodies are supplied in HEPES buffer with 50% glycerol and less than 0.02% sodium azide.**

**Recommended Antibody Dilutions:**
- Western blotting: 1:1000

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

**Products Included**

<table>
<thead>
<tr>
<th>Product #</th>
<th>Quantity</th>
<th>Mol. Wt.</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>8644</td>
<td>20 µl</td>
<td>66 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>5587</td>
<td>20 µl</td>
<td>66 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>2517</td>
<td>20 µl</td>
<td>66 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>2511</td>
<td>20 µl</td>
<td>66 kDa</td>
<td>Mouse IgG2b</td>
</tr>
<tr>
<td>7074</td>
<td>100 µl</td>
<td>66 kDa</td>
<td>Goat</td>
</tr>
<tr>
<td>7076</td>
<td>100 µl</td>
<td>66 kDa</td>
<td>Goat</td>
</tr>
</tbody>
</table>

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

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
   - Prepare fresh 3X reducing loading buffer by adding 1/10 volume 3X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 1X running buffer to 900 ml dH₂O, mix.
5. **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.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 1X TBST to 900 ml dH₂O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **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.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **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.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:**
16. **Detection Reagent:**
   - LumiGLO® chemiluminescent reagent and peroxide (#7003) or
e luminescent reagent and peroxide (#7003) or
   - SignalFire™ (SignalFire™ ECL Reagent (#6883))

B. Protein Blotting

A general protocol for sample preparation.

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 µ per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately accape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 1 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrophoretically transfer 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

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

II. Primary Antibody Incubation

1. 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.
2. Wash three times for 5 min each with 15 ml of TBST.
3. 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.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. 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.
2. 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.