

#9924 Store at -20°C

# Phospho-Estrogen Receptor $\alpha$ Antibody Sampler Kit

✓ 1 Kit  
(4 x 40  $\mu$ l)



**Orders** ■ 877-616-CELL (2355)  
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Estrogen Receptor $\alpha$ (62A3) Mouse mAb	2512	40 $\mu$ l	66 kDa	Mouse IgG2a
Phospho-Estrogen Receptor $\alpha$ (Ser167) (D1A3) Rabbit mAb	5587	40 $\mu$ l	66 kDa	Rabbit IgG
Phospho-Estrogen Receptor $\alpha$ (Ser104/106) Antibody	2517	40 $\mu$ l	66 kDa	Rabbit IgG
Phospho-Estrogen Receptor $\alpha$ (Ser118) (16J4) Mouse mAb	2511	40 $\mu$ l	66 kDa	Mouse IgG2b
Anti-rabbit IgG, HRP-linked Antibody	7074	100 $\mu$ l		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 $\mu$ l		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

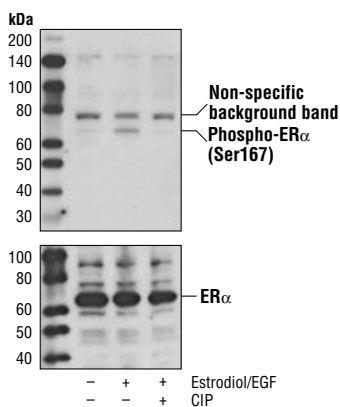
**Description:** The Phospho-Estrogen Receptor  $\alpha$  Antibody Sampler Kit provides an economical means to evaluate the activation status of ER $\alpha$ , including phosphorylation of Ser104/106 and Ser118. The monoclonal control ER $\alpha$  antibody is also included. The phospho-specific monoclonal is included as an alternative. The kit contains enough primary and secondary antibodies to perform four mini-blot experiments.

**Background:** Estrogen receptor  $\alpha$  (ER  $\alpha$ ), 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), ER  $\alpha$  regulates transcription by recruiting coactivator proteins and interacting with general transcriptional machinery (2). Phosphorylation provides an important mechanism to regulate ER  $\alpha$  activity (3,4). ER  $\alpha$  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 ER  $\alpha$  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  $\alpha$  (Ser104/106) Antibody #2517 detects endogenous levels of Ser104/106 phosphorylated ER $\alpha$ . Phospho-Estrogen Receptor  $\alpha$  (Ser118) (16J4) Mouse mAb #2511 will detect endogenous levels of Ser118 phosphorylated ER $\alpha$ . Estrogen Receptor  $\alpha$  (62A3) Mouse mAb #2512 detects endogenous levels of ER $\alpha$ . Phospho-Estrogen Receptor  $\alpha$  (Ser167) (D1A3) Rabbit mAb detects endogeneous levels of ER $\alpha$  protein only when phosphorylated at Ser167, and also cross reacts with a nonspecific band at around 77 kDa. Each antibody in the kit does not cross-react with phosphorylated or nonphosphorylated ER isoforms  $\beta$  or other related family members.

**Source/Purification:** Polyclonal antibody #2517 is produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser104/106 of human ER $\alpha$ . Polyclonal antibodies are purified by protein A and peptide affinity chromatography. The monoclonal antibodies Estrogen Receptor  $\alpha$  (62A3) Mouse mAb and Phospho-Estrogen Receptor  $\alpha$  (Ser118) (16J4) Mouse mAb are produced by immunizing mice with synthetic non-phospho- and phosphopeptides corresponding to residues surrounding Ser118 of human ER $\alpha$ , respectively.

Phospho-Estrogen Receptor  $\alpha$  (Ser167) Rabbit mAb is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser167 of human estrogen receptor  $\alpha$  protein.



Western analysis of extracts from MCF7 cells, untreated or treated with Estrodiol/EGF (100nM each, together for 30 min) and further treated with calf intestinal phosphatase (CIP), using Phospho-Estrogen Receptor  $\alpha$  (Ser167) (D1A3) Rabbit mAb #5587 (upper) or Estrogen Receptor  $\alpha$  (D62A3) Mouse mAb #2512 (lower).

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. Store at  $-20^{\circ}$ C. Do not aliquot the antibodies.

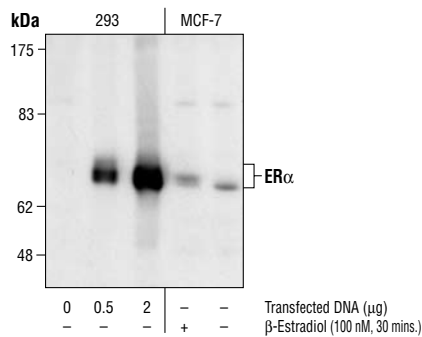
Monoclonal antibodies are supplied in HEPES buffer with 50% glycerol and less than 0.02% sodium azide.

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

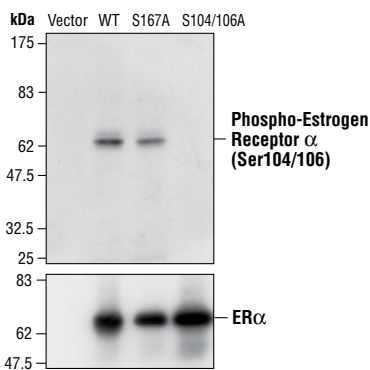
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

### Background References:

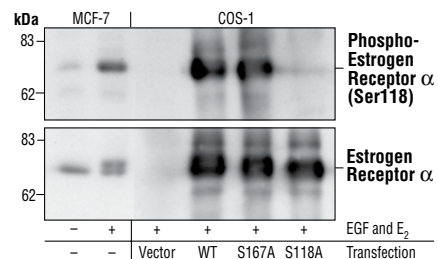
- (1) Mangelsdorf, D.J. et al. (1995) *Cell* 83, 835–839.
- (2) Glass, C.K. and Rosenfeld, M.G. (2000) *Genes Dev.* 14, 121–141.
- (3) Chen, D. et al. (1999) *Mol. Cell. Biol.* 19, 1002–1015.
- (4) Campbell, R.A. et al. (2001) *J. Biol. Chem.* 276, 9817–9824.
- (5) Chen, D. et al. (2000) *Mol. Cell* 6, 127–137.
- (6) Joel, P.B. et al. (1998) *Mol. Cell. Biol.* 18, 1978–1984.



Western blot analysis of extracts from 293 cells expressing wild-type ER  $\alpha$ , and untransfected MCF-7 cells using **Estrogen Receptor  $\alpha$  (62A3) Mouse mAb #2512**.



Western blot analysis of extracts from COS-1 cells expressing wild-type or mutant ER  $\alpha$ , stimulated with  $\beta$ -estradiol (100 nM) and EGF (100 ng/ml) for 30 minutes, using **Phospho-Estrogen Receptor  $\alpha$  (Ser104/106) Antibody #2517** (upper) or control **Estrogen Receptor  $\alpha$  (62A3) Mouse mAb #2512** (lower). (Cell lysates provided by Dr. Simak Ali, Hammersmith Hospital, London.)



Western blot analysis of extracts from untransfected MCF-7 cells, and COS-1 cells transfected with wild-type or mutant ER  $\alpha$ , stimulated with EGF and E<sub>2</sub> using **Phospho-Estrogen Receptor  $\alpha$  (Ser118) (16J4) Mouse mAb #2511** (upper) or control **Estrogen Receptor  $\alpha$  (62A3) Mouse mAb #2512** (lower).

## 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<sup>®</sup>-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<sup>®</sup> 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 prestained 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.

## Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- 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 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

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

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- 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.

- 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.