Store at -20°C

#5587

Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb

☑ 100 µl
(10 western blots)

Cell Signaling TECHNOLOGY®

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New 09/10

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

Entrez-Gene ID #2099
Swiss-Prot Acc. #P03372

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W Endogenous	H, (Mk)	66 kDa	Rabbit IgG**

Background: Estrogen receptor α (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). Ser104, 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).

Specificity/Sensitivity: Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb detects endogeneous levels of ERα protein only when phosphorylated at Ser167. The antibody cross reacts with a nonspecific band at around 77 kDa.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser167 of human estrogen receptor α protein.

Background References:

(1) Mangelsdorf, D.J. et al. (1995) *Cell* 83, 835-839.

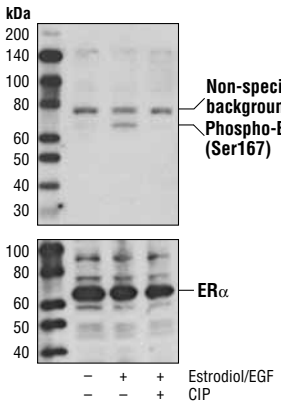
(2) Glass, C.K. and Rosenfeld, M.G. (2000) *Genes Dev.* 14, 121-141.

(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 MCF7 cells, untreated or treated with Estrodiol/EGF (100 nM each, together for 30 min) and further treated with calf intestinal phosphatase (CIP), using Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb (upper) or Estrogen Receptor α (D62A3) Mouse mAb (lower).

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 antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

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

IMPORTANT: 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.

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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Estrogen Receptor α (D8H8) Rabbit mAb

☑ 100 µl
(10 western blots)

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Swiss-Prot Acc. #P03372

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, ChIP	H	66 kDa	Rabbit IgG**
Endogenous			

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 antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

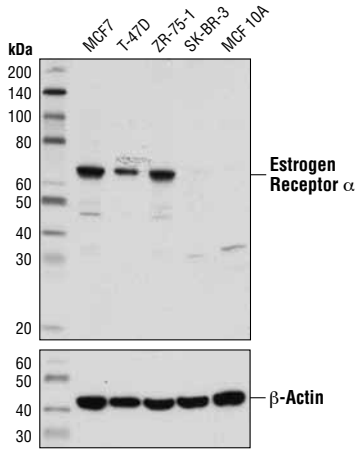
Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:3200
Chromatin IP	1:100

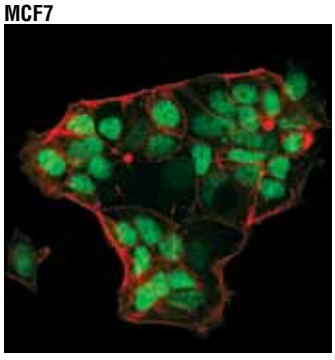
For product specific protocols please see the web page for this product at www.cellsignal.com.

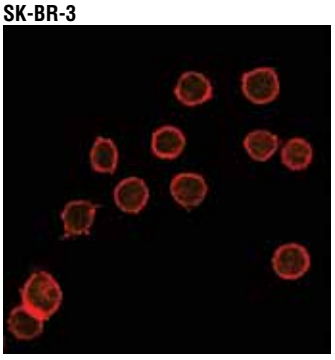
Please visit www.cellsignal.com for a complete listing of recommended complementary products.

Background: Estrogen receptor α (ERα), a member of the steroid receptor superfamily, contains highly conserved DNA binding and ligand binding domains (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 at multiple sites provides an important mechanism to regulate ERα activity (3-5). Ser104, 106, 118, and 167 are located in the amino-terminal transcription activation function domain AF-1, and phosphorylation of these serine residues 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). According to the research literature, phosphorylation at Ser167 may confer tamoxifen resistance in breast cancer patients (4).



Western blot analysis of extracts from ER-positive cell lines (MCF7, T-47D, ZR-75-1) and ER-negative cell lines (SK-BR-3 and MCF 10A) using Estrogen Receptor α (D8H8) Rabbit mAb (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).





◀ *Confocal immunofluorescent analysis of MCF7 (upper) or SK-BR-3 (lower) cells using Estrogen Receptor α (D8H8) Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).*

Specificity/Sensitivity: Estrogen Receptor α (D8H8) Rabbit mAb recognizes endogenous levels of total ERα protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues in the carboxy terminus of human ERα protein.

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.
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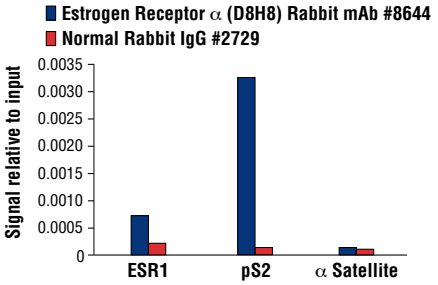

Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 MCF7 cells grown in phenol red free medium and 5% charcoal stripped FBS for 4 d then treated with β-estradiol (10 nM) for 1 h and either 5 µl of Estrogen Receptor α (D8H8) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human ESR1 Promoter Primers #9673, SimpleChIP® Human pS2 Promoter Primers #9702, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

IMPORTANT: 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.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
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