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). Ser106, 108, 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).

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:

Recommended Antibody Dilutions:
Western blotting 1:1000
Immunoprecipitation 1:50
Chromatin IP / Chromatin IP-seq 1:100
Optimal ChIP / ChIP-seq conditions: 5 µl of antibody & 10 µg of chromatin (4 x 10^6 cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

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 (D68B) Rabbit mAb #8457 (lower).

Chromatin immunoprecipitations were performed with cross-linked chromatin from MCF7 cells grown in phenol red free medium and 5% charcoal stripped FBS for 4 d then treated with 10nM estradiol (10 nM) for 45 minutes and either Estrogen Receptor α (D8H8) Rabbit mAb or 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 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.

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

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

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.
Chromatin immunoprecipitations were performed with cross-linked chromatin from MCF7 cells grown in phenol red free medium and 5% charcoal stripped FBS for 4 d then treated with β-estradiol (10 nM) for 45 minutes and 5 µl of Estrogen Receptor α (D8H8) Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared using Simple-ChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 21 (upper), including TFF1/pS2 (lower), a known target gene of Estrogen Receptor α (see additional figure containing ChIP-qPCR data).