Anti-Phospho-Estrogen Receptor alpha (S118) Antibody [SC05-87]

ET1610-32

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 66 kDa
Clone number:	SC05-87

Description: Estrogen receptor alpha (ERa, ER, ESR, ESRA, Era, NR3A1, estrogen receptor 1) is a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding and activation of transcription. Alternative splicing results in several ERa mRNA transcripts, which differ primarily in their 5' untranslated regions. ERa undergoes phosphorylation in response to estradiol binding. Human ERa is predominately phosphorylated on Ser 118 and to a lesser extent on Ser 104 and Ser 106. In response to activation of the mitogen-activated protein kinase pathway, phosphorylation occurs on Ser 118 and Ser 167. These serine residues are all located within the activation function 1 region of the N-terminal domain of ERa. In contrast, activation of protein kinase A increases the phosphorylation of Ser 236, which is located in the DNA-binding domain. Src kinase-dependent Tyr 537 phosphorylation may enhance estrogen binding to ERa. Mutation of Tyr 537 of the human ERa produces receptors having a range of constitutive activity.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser118 of Human ESR1 aa 110-154 / 595.

Positive control: MCF7 whole cell lysate, MCF7 treated with 100nM Estradiol for 30 minutes add 100ng/ml EGF for 5 minutes whole cell lysate, MCF7 treated with 100nM Estradiol for 30 minutes then treated with 100ng/ml EGF for 5 minutes whole cell lysate, Hela, MCF-7, HUVEC, human breast carcinoma tissue, human breast tissue, mouse placenta tissue, human placenta tissue, human fallopian tube tissue, human uterus tissue.

Subcellular location: Nucleus, Cytoplasm, Cell membrane.

Database links: SwissProt: P03372 Human | P19785 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

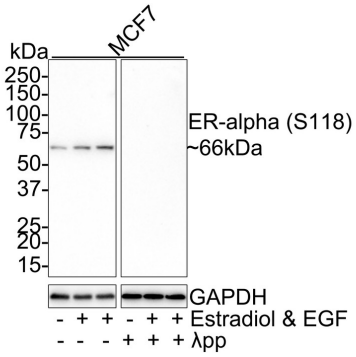

Fig1: Western blot analysis of Phospho-Estrogen Receptor alpha (S118) on different lysates with Rabbit anti-Phospho-Estrogen Receptor alpha (S118) antibody (ET1610-32) at 1/1,000 dilution.

Lane 1: MCF7 whole cell lysate
Lane 2: MCF7 treated with 100nM Estradiol for 30 minutes add 100ng/ml EGF for 5 minutes whole cell lysate
Lane 3: MCF7 treated with 100nM Estradiol for 30 minutes then treated with 100ng/ml EGF for 5 minutes whole cell lysate
Lane 4: MCF7 whole cell lysate treated with λpp for 1 hour
Lane 5: MCF7 treated with 100nM Estradiol for 30 minutes add 100ng/ml EGF for 5 minutes whole cell lysate treated with λpp for 1 hour
Lane 6: MCF7 treated with 100nM Estradiol for 30 minutes then treated with 100ng/ml EGF for 5 minutes whole cell lysate treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 66 kDa
Observed band size: 66 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-32) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

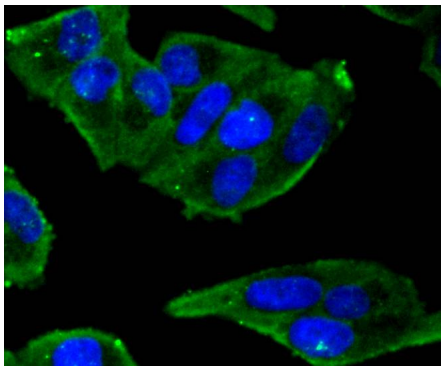

Fig2: ICC staining of Phospho-Estrogen Receptor alpha (S118) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-32, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

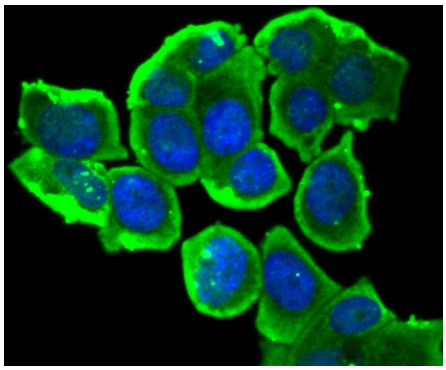

Fig3: ICC staining of Phospho-Estrogen Receptor alpha (S118) in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-32, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

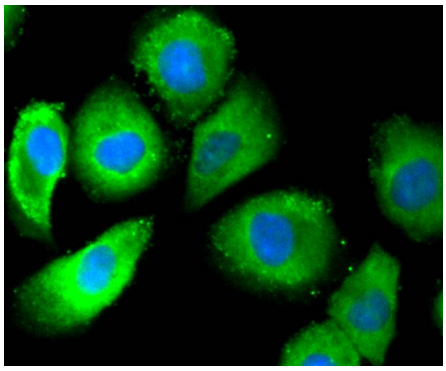

Fig4: ICC staining of Phospho-Estrogen Receptor alpha (S118) in HUVEC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-32, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

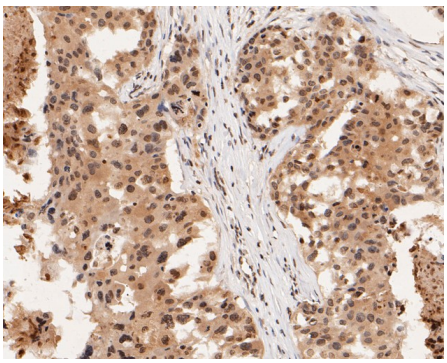

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-Estrogen Receptor alpha (S118) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-32, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

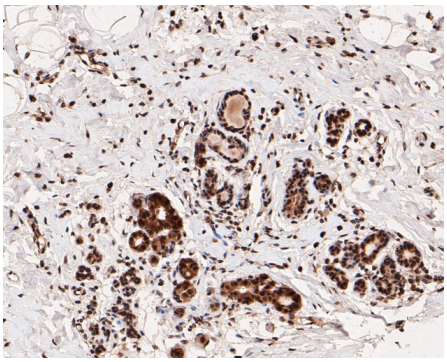

Fig6: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-Phospho-Estrogen Receptor alpha (S118) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-32, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

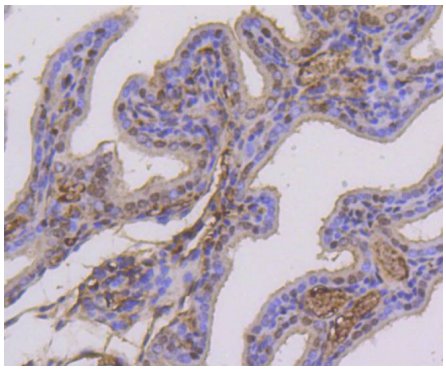

Fig7: Immunohistochemical analysis of paraffin-embedded mouse placenta tissue using anti-Phospho-Estrogen Receptor alpha (S118) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-32, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

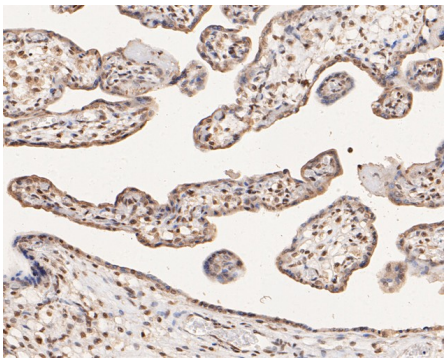

Fig8: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-Phospho-Estrogen Receptor alpha (S118) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-32, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

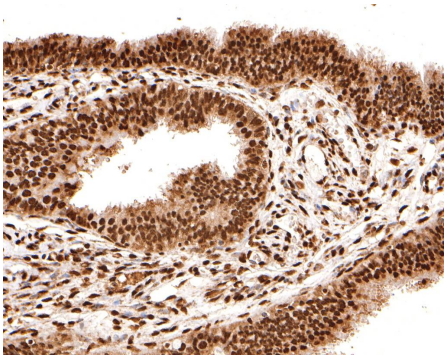

Fig9: Immunohistochemical analysis of paraffin-embedded human fallopian tube tissue using anti-Phospho-Estrogen Receptor alpha (S118) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-32, 1/400) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

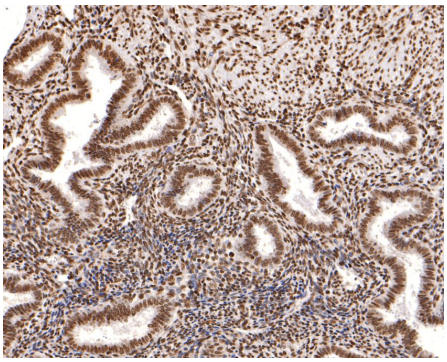

Fig10: Immunohistochemical analysis of paraffin-embedded human uterus tissue using anti-Phospho-Estrogen Receptor alpha (S118) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-32, 1/400) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Moriel-Carretero M et al. Control of the function of the transcription and repair factor TFIIH by the action of the cochaperone Ydj1. Proc Natl Acad Sci U S A 108:15300-5 (2011).
2. Hennessy BT et al. A Technical Assessment of the Utility of Reverse Phase Protein Arrays for the Study of the Functional Proteome in Non-microdissected Human Breast Cancers. Clin Proteomics 6:129-51 (2010).