Human ERα/NR3A1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF5715

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human ERα.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human ERα Met1-Gln116 Accession # P03372
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

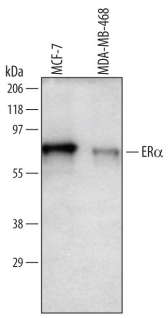
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	5 µg/mL	See Below

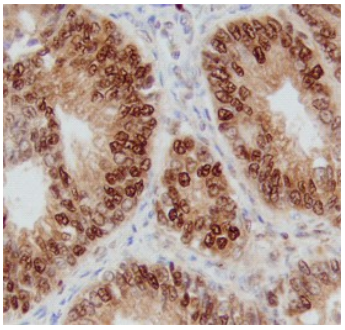
DATA

Western Blot



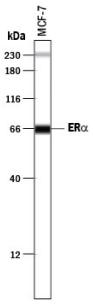
Detection of Human ERα/NR3A1 by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line and MDA-MB-468 human breast cancer cell line. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human ERα/NR3A1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5715) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for ERα/NR3A1 at approximately 65 to 70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



ERα/NR3A1 in Human Endometrial Cancer Tissue. ERα/NR3A1 was detected in immersion fixed paraffin-embedded sections of human endometrial cancer tissue using Sheep Anti-Human ERα/NR3A1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5715) at 10 µg/mL overnight at 4 °C. Before incubation with the primary antibody tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human ERα/NR3A1 by Simple Western™. Simple Western lane view shows lysates of MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for ERα/NR3A1 at approximately 66 kDa (as indicated) using 5 µg/mL of Sheep Anti-Human ERα/NR3A1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5715) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

ERα (Estrogen receptor alpha; also Estradiol receptor and NR3A1) is a 65-70 kDa member of the NR3 subfamily, nuclear hormone receptor family of proteins. It is widely expressed, and serves as a strong activator of estrogen-responsive genes. ERα is normally quiescent and bound to heat-shock proteins and immunophilins. Following β-estradiol binding, it becomes activated, either homodimerizes or heterodimerizes with ERβ, and binds to DNA with multiple coactivators. Human ERα is 595 amino acids (aa) in length, and contains a DNA binding region (aa 185-250), three NLSs (aa 256-260; 266-271; 299-303), a steroid-binding site (aa 351-543), a dimerization motif (aa 497-518), and an O-GlcNAc attachment around Thr575. Major phosphorylation sites exist at Tyr537, Ser167 and Ser118.

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