

Product datasheet

Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade ab32063

Recombinant RabMAb

★★★★☆ 17 Abreviews 47 References 14 Images

Overview

Product name	Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade
Description	Rabbit monoclonal [E115] to Estrogen Receptor alpha - ChIP Grade
Host species	Rabbit
Specificity	Expression levels of ER alpha protein vary with sample type. ab32063 is unsuitable to test ovary and the tissues with low expression level of Estrogen Receptor alpha, such as kidney, lung and brain, in western blot. And it failed to show good IHC signal on mouse and rat tissue sections when using our IHC testing conditions. For our in-house testing we tested the antibody on a mouse tissue array (breast, spleen, lung, stomach, muscle, pancreas, liver, colon, brain, kidney).
Tested applications	Suitable for: ICC/IF, Flow Cyt, ChIP, WB, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Estrogen Receptor alpha aa 50-150. The exact sequence is proprietary. Database link: P03372 (Peptide available as ab203371)
Positive control	WB: MCF-7 and T47-D cell lysates. IHC-P: Human breast carcinoma and endometrial carcinoma tissues; human endometrium and breast tissues. ICC/IF: MCF-7 cells. Flow Cyt: MCF-7 cells.
General notes	Our RabMAb [®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents . We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team. This product is a recombinant rabbit monoclonal antibody .

Properties

Form Liquid

Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E115
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab32063** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/200.
Flow Cyt	★★★★★	1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. We recommend to use a 30 min blocking step (1XPBS/10%goat serum/0,3M Glycin).
ChIP	★★★★★	Use 4 µg for 25 µg of chromatin.
WB	★★★★★	1/1000. Detects a band of approximately 60 kDa (predicted molecular weight: 67 kDa).Can be blocked with Estrogen Receptor alpha peptide (ab203371) .
IHC-P	★★★★★	1/200 - 1/5000. For unpurified, use 1/50 - 1/100. The antibody failed to show good IHC signal on mouse and rat tissue sections when applied using our IHC testing conditions.

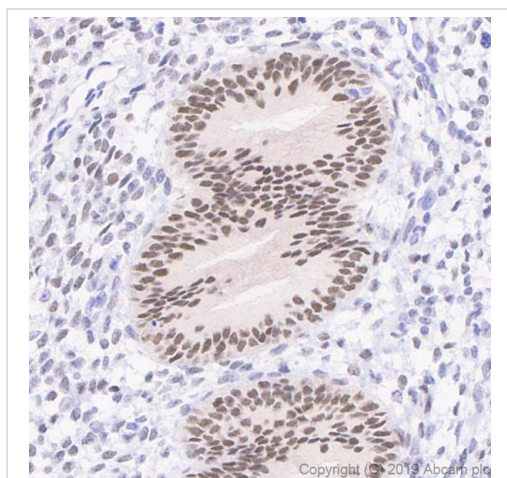
Target

Function	Nuclear hormone receptor. The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues. Can activate the transcriptional activity of TFF1.
Sequence similarities	Belongs to the nuclear hormone receptor family. NR3 subfamily. Contains 1 nuclear receptor DNA-binding domain.
Domain	Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.
Post-translational modifications	Phosphorylated by cyclin A/CDK2. Phosphorylation probably enhances transcriptional activity. Glycosylated; contains N-acetylglucosamine, probably O-linked. Ubiquitinated. Deubiquitinated by OTUB1. Dimethylated by PRMT1 at Arg-260. The methylation may favor cytoplasmic localization. Palmitoylated (isoform 3). Not biotinylated (isoform 3).

Cellular localization

Nucleus. Cytoplasm. Cell membrane. A minor fraction is associated with the inner membrane and Nucleus. Cytoplasm. Cell membrane. Associated with the inner membrane via palmitoylation.

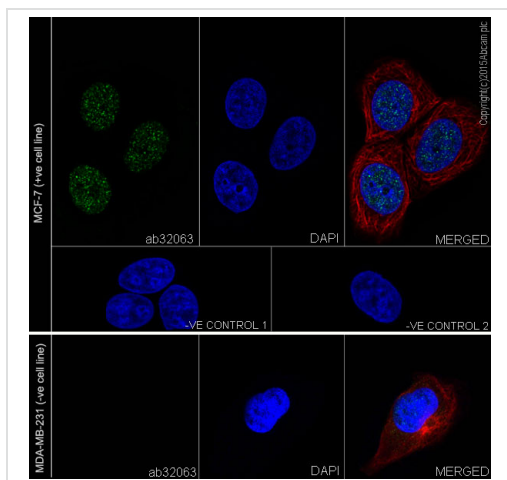
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunohistochemical staining of paraffin embedded human endometrium tissue with ab32063 at a dilution of 1/5000. The secondary antibody used was Goat Anti-Rabbit IgG H&L (HRP Polymer). The sample is counter-stained with hematoxylin. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0).

Nuclear staining on human endometrium.

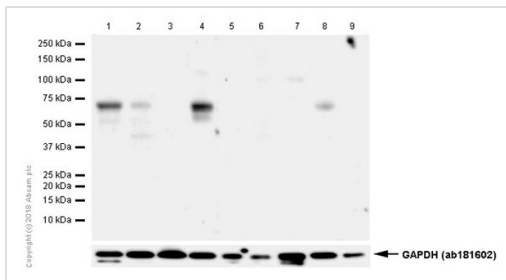


Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling Estrogen Receptor alpha with purified ab32063 at 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with ab7291, a mouse anti-tubulin (1/1000) using ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).

Control 1: primary antibody (1/1000) and secondary antibody, ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000).



Western blot - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

All lanes : Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063) at 1/200 dilution

Lane 1 : Human uterus tissue lysates

Lane 2 : Human kidney tissue lysates

Lane 3 : Human brain tissue lysates

Lane 4 : Mouse uterus tissue lysates

Lane 5 : Mouse ovary tissue lysates

Lane 6 : Mouse kidney tissue lysates

Lane 7 : Mouse brain tissue lysates

Lane 8 : Rat uterus tissue lysates

Lane 9 : Rat ovary tissue lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

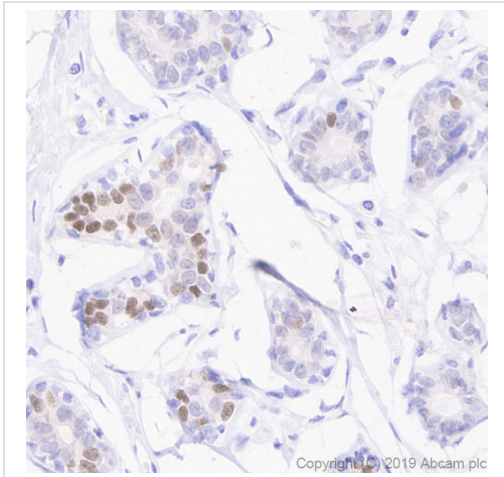
Predicted band size: 67 kDa

Observed band size: 67 kDa

Exposure time: 180 seconds

Blocking and diluting buffer: 5% NFDM/TBST.

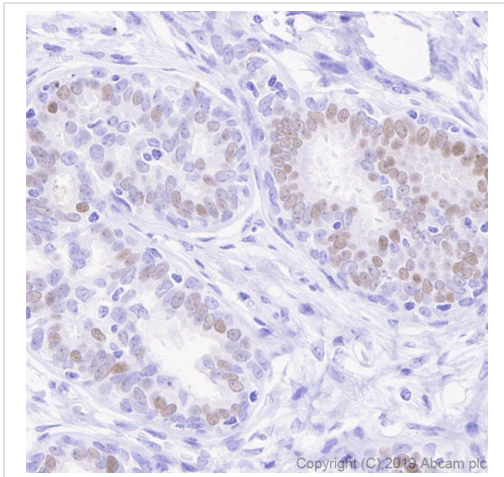
The expression level of ER66 is high in uterus, moderate in ovary and low in kidney, lung, brain, liver, heart (PMID: 20861365, 24977106, 23675257, 23940668, 22070562), especially low in the tissues from male or young female animals (PMID: 26384003, 23940668). ab32063 is unsuitable to test ovary and the tissues with low expression level of Estrogen Receptor alpha in western blot.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunohistochemical staining of paraffin embedded human breast tissue with ab32063 at a dilution of 1/5000. The secondary antibody used was Goat Anti-Rabbit IgG H&L (HRP Polymer). The sample is counter-stained with hematoxylin. Antigen retrieval was heat mediated using [ab93684](#) (Tris/EDTA buffer, pH 9.0).

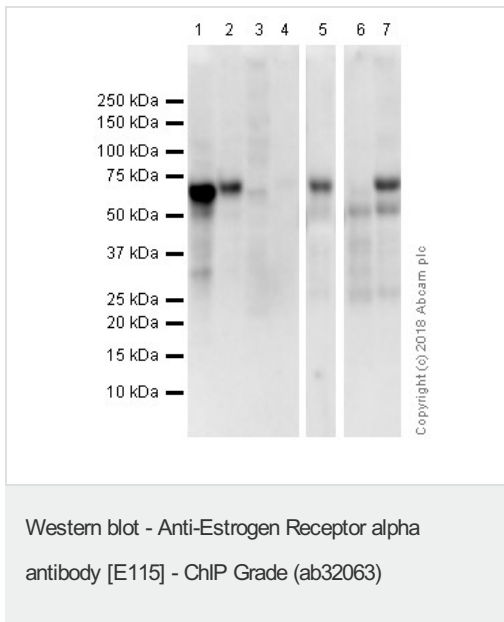
Nuclear staining on human breast.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunohistochemical staining of paraffin embedded human breast carcinoma tissue with ab32063 at a dilution of 1/5000. The secondary antibody used was Goat Anti-Rabbit IgG H&L (HRP Polymer). The sample is counter-stained with hematoxylin. Antigen retrieval was heat mediated using [ab93684](#) (Tris/EDTA buffer, pH 9.0).

Nuclear staining on human breast carcinoma.



All lanes : Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063) at 1/1000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell). Whole cell lysates

Lane 2 : T-47D (human mammary gland ductal carcinoma epithelial cell). Whole cell lysates

Lane 3 : MDA-MB231 (Human breast adenocarcinoma epithelial cell) Whole cell lysates (Negative control)

Lane 4 : HepG2 (Human hepatocellular carcinoma epithelial cell) Whole cell lysates (Negative control)

Lane 5 : Human uterus whole tissue lysate

Lane 6 : Human ovary whole tissue lysate

Lane 7 : Human ovary cancer whole tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

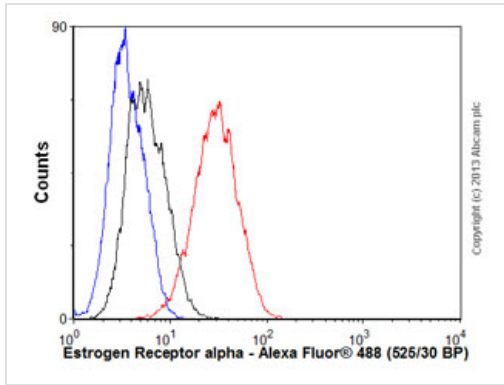
Predicted band size: 67 kDa

Observed band size: 68 kDa

[why is the actual band size different from the predicted?](#)

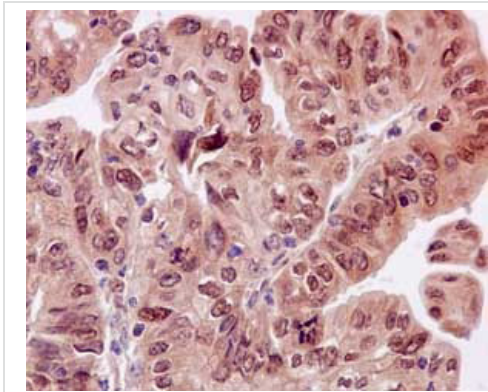
Exposure time: 50 seconds

Blocking and diluting buffer: 5% NFDm/TBST.



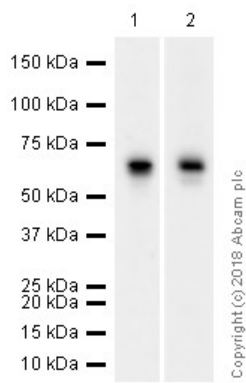
Flow Cytometry - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Overlay histogram showing MCF7 cells stained with unpurified ab32063 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32063, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in MCF7 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunohistochemical staining of paraffin embedded human endometrial carcinoma with purified ab32063 at a working dilution of 1 in 200. The secondary antibody used is ab97051, a HRP goat anti-rabbit IgG (H+L), at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.



Western blot - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

All lanes : Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063) at 1/1000 dilution

Lane 1 : Rat pituitary whole tissue lysate

Lane 2 : Mouse pituitary whole tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

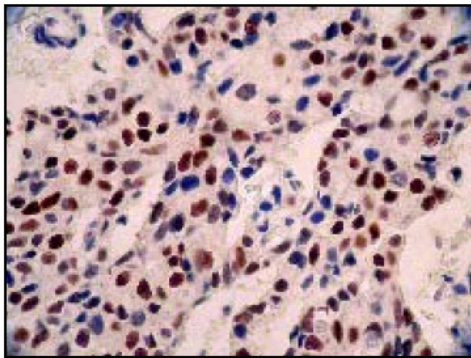
Predicted band size: 67 kDa

Observed band size: 68 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 1st lane: 85 seconds

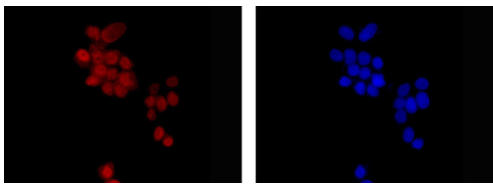
2nd lane: 32 seconds

Blocking and diluting buffer: 5% NFDm/TBST



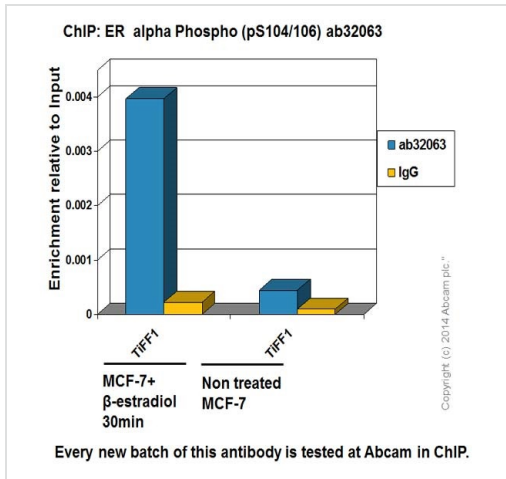
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunohistochemical analysis of human breast carcinoma using anti-Estrogen Receptor alpha (ab32063, unpurified) diluted 1:50



Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunofluorescent staining of MCF7 cells (fixed with 4% PFA and permeabilized with TritonX 100) with purified ab32063 at a dilution of 1/250. An Alexa Fluor® 555 goat anti-rabbit antibody was used as the secondary at a dilution of 1/200. The panel on the right shows the DAPI counter-staining.



ChIP - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Chromatin was prepared from MCF-7+ β -estradiol 30 min, and MCF-7 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 μ g of chromatin, 4 μ g of purified ab32063 (blue), and 20 μ LI of anti-rabbit IgG sepharose beads. Rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (SYBR approach). Primers are located in the 2nd intron of TFF1 gene.

MCF7 Cells were treated as below:

MCF-7 starved overnight, then treated with 10 nM β -Estradiol in 2% FBS media for 30 min.

Control MCF-7 was starved overnight, then in 2% FBS media for 30 mins.

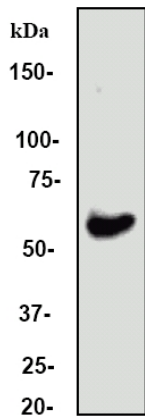
Primer information:

Located to the 2 intron of TFF1 gene.

Sequence:

Forward: 5' -agtctctccaacctgacctt-3'

Reverse: 5' -ttccggccatctctcactat-3'

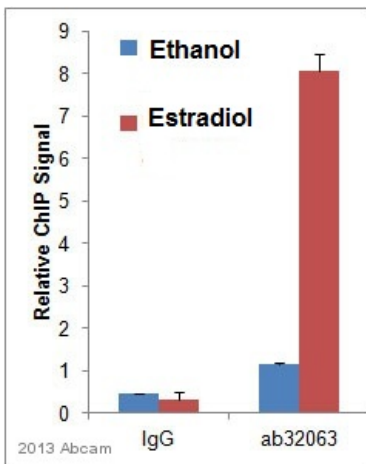


Western blot - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063) at 1/500 dilution (unpurified) + MCF-7 cell lysate

Predicted band size: 67 kDa

Observed band size: 60 kDa [why is the actual band size different from the predicted?](#)



ChIP - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

This image is courtesy of an anonymous Abreview

ChIP analysis using unpurified ab32063 binding Estrogen Receptor alpha in MCF7 cells derived from Human breast carcinoma. Cells were cross-linked for 10 minutes with 1% formaldehyde. Samples were incubated with undiluted primary antibody for 16 hours at 4°C. Protein binding was detected using real-time PCR.

Positive control: Estrogen treated MCF7 cells tested at PS2 promoter.

Negative Control: IgG ChIP and ethanol-depleted cells tested at PS2 promoter.

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