abcam

Product datasheet

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

重组 RabMAb

21 Abreviews 143 References 20 图像

概述

产品名称	Anti-Estrogen Receptor alpha抗体[E115] - ChIP Grade
描述	兔单克隆抗体[E115] to Estrogen Receptor alpha - ChIP Grade
宿主	Rabbit
特异性	Expression levels of ER alpha protein vary with sample type. This antibody 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).
经测试应用	**适用于:** ICC/IF, ChIC/CUT&RUN-seq, ChIP, WB, Flow Cyt (Intra), IHC-P
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab203371**)
阳性对照	WB: MCF-7 and T47-D cell lysates; Human uterus and ovary cancer tissue lysates; Mouse and rat uterus lysates; Rat and mouse pituitary whole tissue lysates. IHC-P: Human breast carcinoma and endometrial carcinoma tissues; human endometrium and breast tissues. ICC/IF: MCF-7 cells, 4T1 cells and GH3 cells. Flow Cyt (intra): MCF-7 cells. ChIP: Chromatin prepared from MCF-7+ÃŸ-estraiol 30 min cells. ChIC/CUT&RUN: MCF7 cells.
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid

存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	E115
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab32063于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

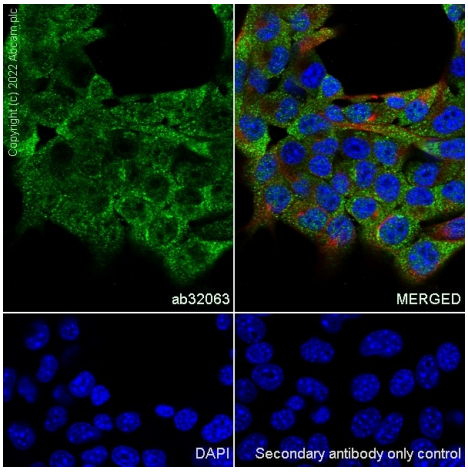
应用	Ab评论	说明
ICC/IF		1/200.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
ChIP	★★★★★ (1)	Use 4 µg for 25 µg of chromatin.
WB	★★★★★ (5)	1/1000. Detects a band of approximately 60 kDa (predicted molecular weight: 67 kDa).
Flow Cyt (Intra)		1/1000. **ab172730** - Rabbit monoclonal IgG, is 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).
IHC-P	★★★★☆ (11)	1/200 - 1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **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.

靶标

功能	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.
序列相似性	Belongs to the nuclear hormone receptor family. NR3 subfamily. Contains 1 nuclear receptor DNA-binding domain.
结构域	Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.

翻译后修饰	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).
细胞定位	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.

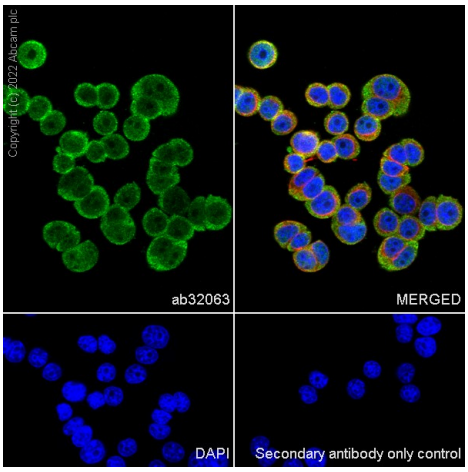
图片



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

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 4T1 (mouse mammary gland carcinoma epithelial cell line) cells labelling Estrogen Receptor alpha with primary antibody anti-Estrogen Receptor alpha (ab32063) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150081**) secondary antibody at 1/1000 dilution (2.0 µg/mL). Confocal image showing cytoplasmic and nuclear staining in 4T1 cells. Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) was used to counterstain tubulin at 1/200 dilution (2.5 µg/mL). The nuclear counter stain is DAPI (blue).

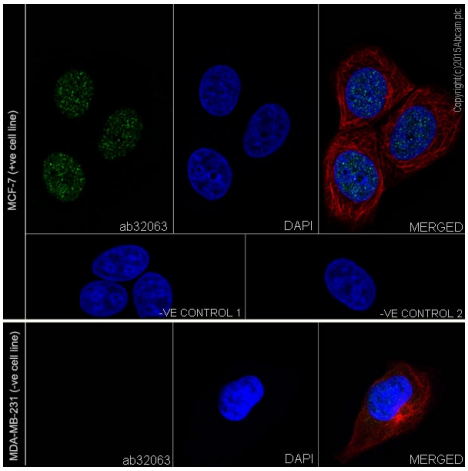
The secondary antibody only control is : Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2.0 µg/mL).



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

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized GH3 (rat pituitary epithelial cell line) cells labelling Estrogen Receptor alpha with primary antibody anti-Estrogen Receptor alpha (ab32063) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150081**) secondary antibody at 1/1000 dilution (2.0 µg/mL). Confocal image showing cytoplasmic and nuclear staining in GH3 cells. Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) was used to counterstain tubulin at 1/200 dilution (2.5 µg/mL). The nuclear counter stain is DAPI (blue).

The secondary antibody only control is : Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2.0 µg/mL).

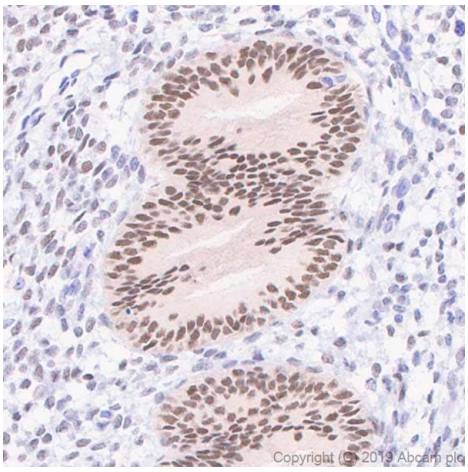


Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

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).

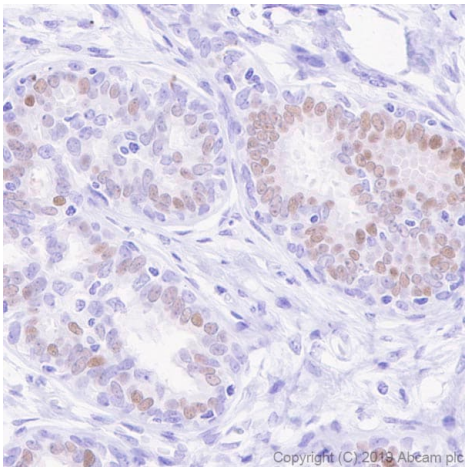
Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



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).

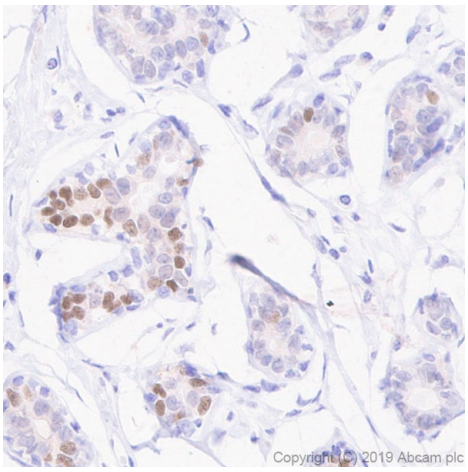
Nuclear staining on human endometrium.



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).

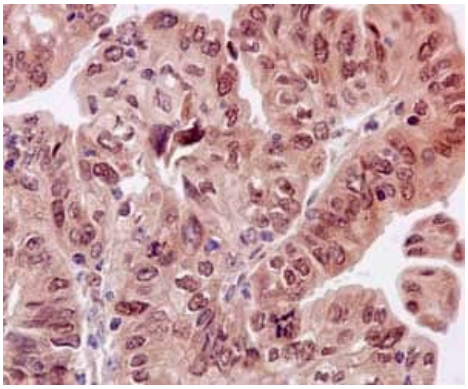
Nuclear staining on human breast carcinoma.



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

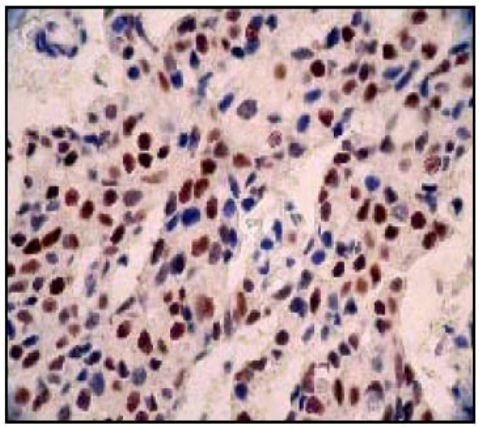
Immunohistochemical staining of paraffin embedded human breast tissue with ab32063 at a dilution of 1/5000. The secondary antibody used was Goat Anti-Rabbit IgG H&L (HRP Polymer). The sample is counter-stained with hematoxylin. Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer,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

Nuclear staining on human breast.



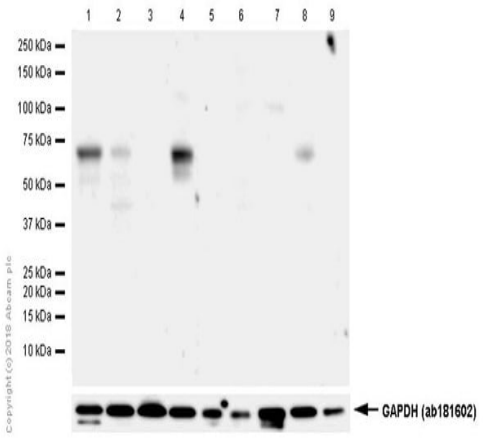
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

Immunohistochemical staining of paraffin embedded human 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 perfomed using Tris-EDTA buffer, pH 9.0.



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



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.

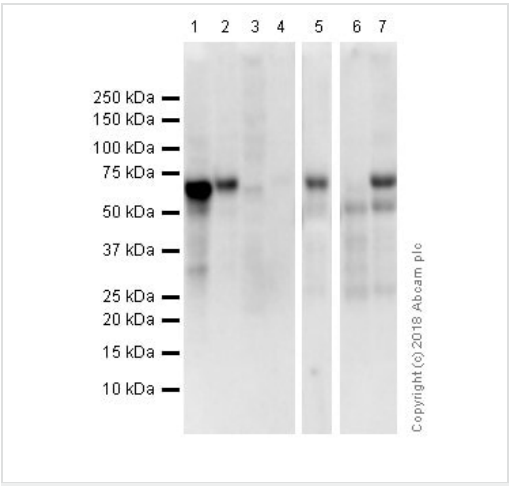
Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

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.



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 : 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

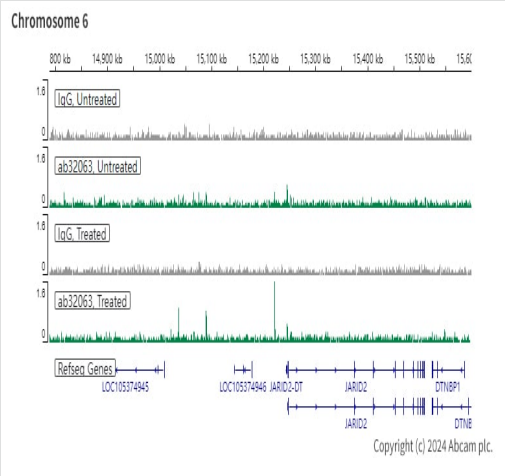
Lysates/proteins at 20 µg per lane.

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

Exposure time: 50 seconds

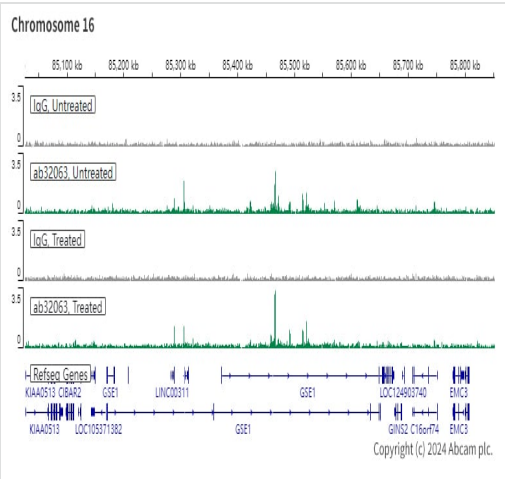
Blocking and diluting buffer: 5% NFDM/TBST.



ChIC/CUT&RUN sequencing - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 MCF7 (Human breast adenocarcinoma epithelial cell) cells treated with phenol red free medium and 5% charcoal stripped FBS for 3 days then treated with β-estradiol (10 nM 45 min) and 5 µg of ab32063 [E115]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

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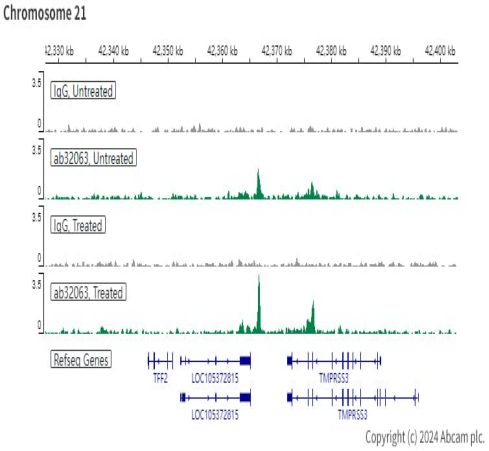
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

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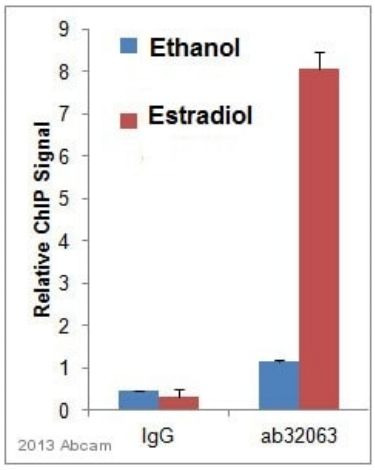
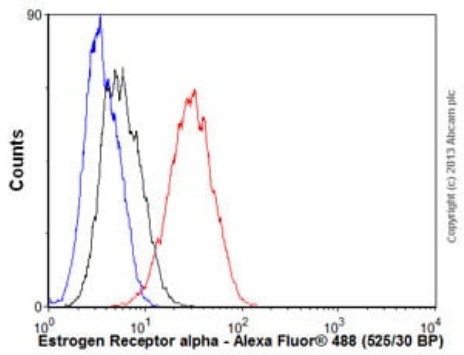
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



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

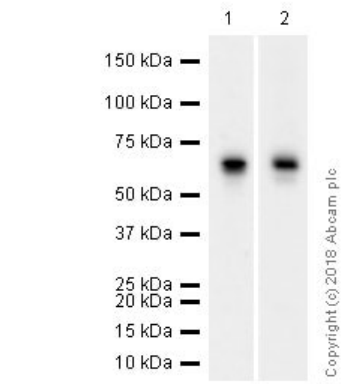
This image is courtesy of an anonymous Abreview

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.



Flow Cytometry (Intracellular) - 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/1x10^6 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.



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

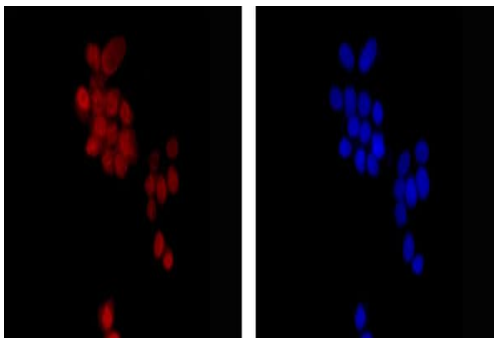
Lysates/proteins at 20 µg per lane.

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

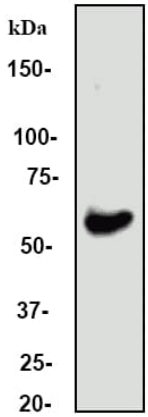
Exposure time: 1st lane: 85 seconds
2nd lane: 32 seconds

Blocking and diluting buffer: 5% NFDM/TBST



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

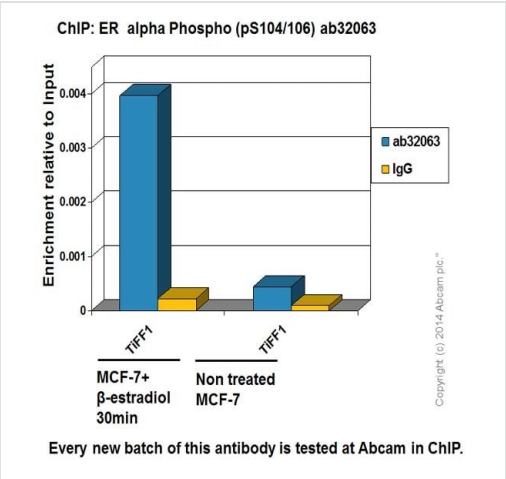
Immunofluorescent staining of MCF7 cells (fixed with 4% PFA and permeablized with TritonX 100) with purified ab32063 at a dilution of 1/250. An Alexa Fluor® 555 goat anti-rabbit antibody was used as the secondary at a dilution of 1/200. The panel on the right shows the DAPI counter-staining.



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



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

Chromatin was prepared from MCF-7+β-estraiol 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 µg of chromatin, 4 µg of purified ab32063 (blue), and 20 µLl 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.

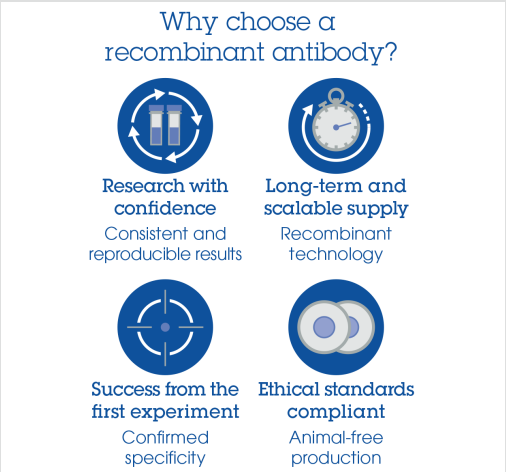
Primer information:

Located to the 2 intron of TFF1 gene.

Sequence:

Forward: 5' -agtctcctccaacctgacctt-3'

Reverse: 5' -ttccggccatctctcactat-3'



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

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- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

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