

# **Product datasheet**

# Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free ab271827

אילשעבע RabMAb

★★★★★ 1 Abreviews 2 References 画像数 16

製品の概要	
製品名	Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free
製品の詳細	Rabbit monoclonal [E115] to Estrogen Receptor alpha - BSA and Azide free
由来種	Rabbit
アプリケーション	適用あり: ChIP, ICC/IF, WB, IHC-P, ChIC/CUT&RUN-seq, Flow Cyt (Intra)
種交差性	交差種: Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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. ChIC/CUT&RUN: MCF7 cells.
特記事項	ab271827 is the carrier-free version of <u>ab32063</u> .
	Our <u><b>carrier-free</b></u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.
	This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.
	Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.
	This product is compatible with the Maxpar <sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar <sup>®</sup> is a trademark of Fluidigm Canada Inc.
	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

## 製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.20 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	E115
アイソタイプ	lgG

## アプリケーション

# **The Abpromise guarantee** Abpromise保証は、次のテスト済みアプリケーションにおけるab271827の使用に適用されます

# アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

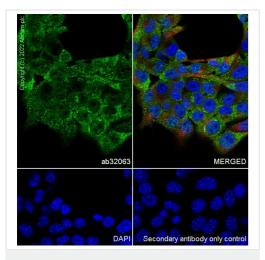
アプリケーション	Abreviews	特記事項
ChIP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 66 kDa.
IHC-P	****(1)	Use at an assay dependent concentration. The antibody failed to show good IHC signal on mouse and rat tissue sections when applied using our IHC testing conditions.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab172730</u> - 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).

## ターゲット情報

機能	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.
配列類似性	Belongs to the nuclear hormone receptor family. NR3 subfamily. Contains 1 nuclear receptor DNA-binding domain.

ドメイン	Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C- terminal ligand-binding domain.
翻訳後修飾	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).
細胞内局在	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.

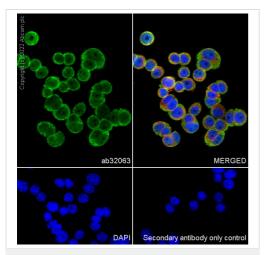
#### 画像



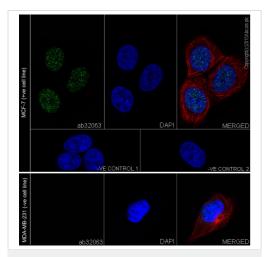
Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 4T1 (mouse mammary gland carcinoma epithelial cell line) cells labelling Estrogen Receptor alpha with primary antibody anti-Estrogen Receptor alpha (**ab32063**) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 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<sup>®</sup> 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 <u>**ab150081**</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution (2.0  $\mu$ g/mL).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32063</u>).



Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)



Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 488) at 1/1000 dilution (2.0 µg/mL).

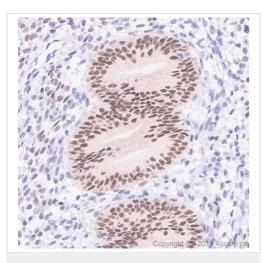
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32063</u>).

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)

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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)

Immunohistochemical staining of paraffin embedded human endometrium tissue with <u>ab32063</u> 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 <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

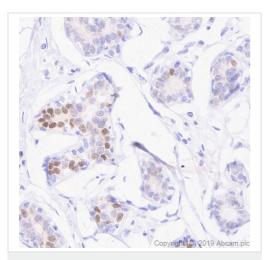
Nuclear staining on human endometrium.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).

Immunohistochemical staining of paraffin embedded human breast carcinoma tissue with <u>ab32063</u> 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 <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

Nuclear staining on human breast carcinoma.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32063</u>).

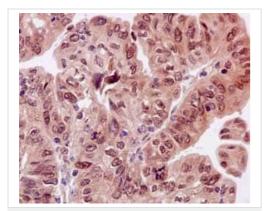


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)

Immunohistochemical staining of paraffin embedded human breast tissue with <u>ab32063</u> 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 <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

Nuclear staining on human breast.

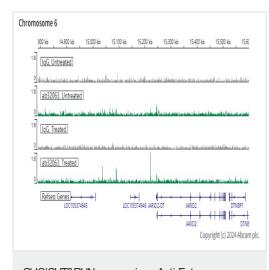
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).



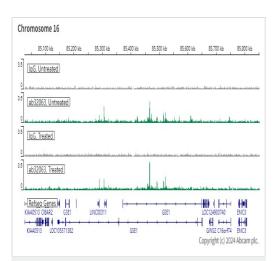
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)

Immunohistochemical staining of paraffin embedded human endometrial carcinoma with purified <u>ab32063</u> at a working dilution of 1 in 200. The secondary antibody used is <u>ab97051</u>, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32063</u>).



ChIC/CUT&RUN sequencing - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)



ChIC/CUT&RUN sequencing - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL,  $2.5 \times 10^{5}$  MCF7 (Human breast adenocarcinoma epithelial cell) cells treated with phenol red free medium and 5% charcoal stripped FBS for 3 days then treated with  $\beta$ -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.

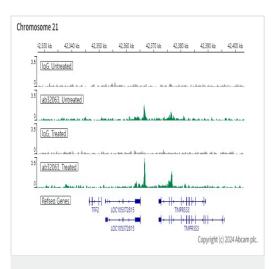
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation (<u>ab32063</u>).

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  $\beta$ -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.

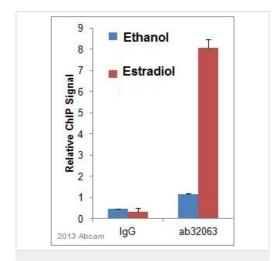
This data was developed using the same antibody clone in a different buffer formulation (<u>ab32063</u>).



ChIC/CUT&RUN sequencing - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) 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  $\beta$ -estradiol (10 nM 45 min) and 5  $\mu$ g 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.

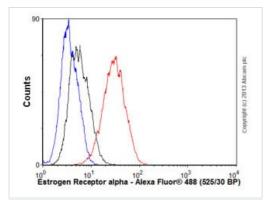
This data was developed using the same antibody clone in a different buffer formulation (<u>ab32063</u>).



ChIP - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) ChIP analysis using unpurified <u>ab32063</u> 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.

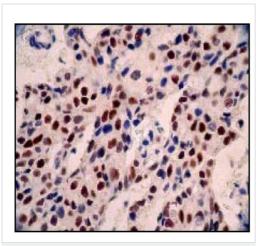
Negative Control:lgG ChIP and ethanol-depleted cells tested at PS2 promoter.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).

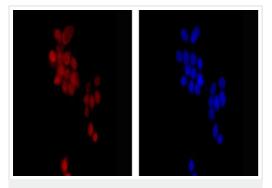


Flow Cytometry (Intracellular) - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) Overlay histogram showing MCF7 cells stained with unpurified ab32063 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. 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<sup>®</sup> 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)  $(1\mu g/1x10^6$  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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32063</u>).



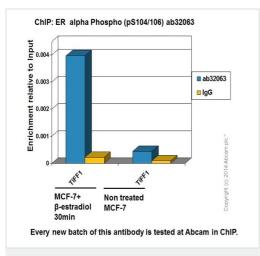
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) Immunohistochemical analysis of human breast carcinoma using anti-Estrogen Receptor alpha (**ab32063**, unpurified) diluted 1:50 This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).



Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827)

Immunofluorescent staining of MCF7 cells (fixed with 4% PFA and permeablized with TritonX 100) with purified <u>ab32063</u> at a dilution of 1/250. An Alexa Fluor<sup>®</sup> 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).



ChIP - Anti-Estrogen Receptor alpha antibody [E115] - BSA and Azide free (ab271827) Chromatin was prepared from MCF-7+ $\beta$ -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 µLI of antirabbit 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  $\beta\text{-}Estradiol$  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'

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32063**).



Anti-Estrogen Receptor alpha antibody [E115] - BSA

and Azide free (ab271827)

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