abcam

Product datasheet

Anti-Estrogen Receptor alpha antibody [EPR4097] ab108398

Recombinant RabMAb

Overview

Product name Anti-Estrogen Receptor alpha antibody [EPR4097]

Description Rabbit monoclonal [EPR4097] to Estrogen Receptor alpha

Host species Rabbit

Specificity Expression levels of ER alpha protein vary with sample type.

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, IHC-Fr, ChIC/CUT&RUN-seq, ICC/IF

Species reactivity Reacts with: Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: MCF7 and T47-D cell lysates. IHC-Fr: Frozen Human cervix and uterus tissue sections. IHC-

P: Human breast ductal infiltrating carcinoma and normal breast tissues. ICC/IF: MCF-7 cells.

General notesThis 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**.

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.

Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity Protein A purified

Clonality Monoclonal

1

Clone number EPR4097

Isotype IgG

Applications

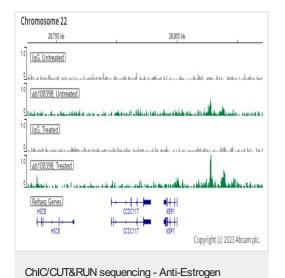
The Abpromise guarantee Our Abpromise guarantee covers the use of ab108398 in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	★★★★★ (<u>5</u>)	1/1000 - 1/10000. Predicted molecular weight: 66 kDa. For unpurified use at 1/200 - 1/1000.
IHC-P	★★★★★ (3)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/30.
IHC-Fr		Use a concentration of 5 µg/ml.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF		1/250. For unpurified use at 1/30.

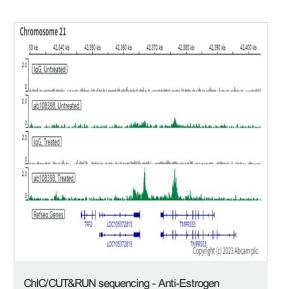
Target		
Function	Nuclear hormone receptor. The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues. Can activate the transcriptional activity of TFF1.	
Sequence similarities	Belongs 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



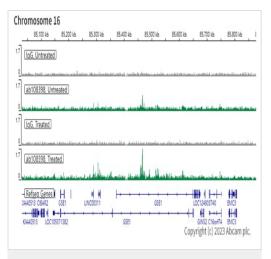
Receptor alpha antibody [EPR4097] (ab108398)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5×10^{5} MCF7 (Human breast adenocarcinoma epithelial cell) cells treated with phenol red free medium and 5% charcoal stripped FBS for 3 days than treated with β -estradiol (10 nM 45 min) and 5 μ g of ab108398 [EPR4097]. 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.



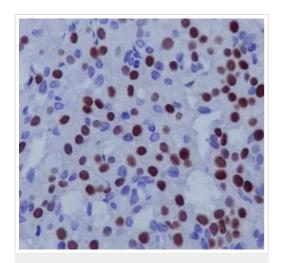
ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/ μ L, 2.5 x 10^5 MCF7 (Human breast adenocarcinoma epithelial cell) cells treated with phenol red free medium and 5% charcoal stripped FBS for 3 days than treated with β -estradiol (10 nM 45 min) and 5 μ g of ab108398 [EPR4097]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control <u>ab172730</u> 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 [EPR4097] (ab108398)

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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast tissue labelling Estrogen Receptor alpha with purified ab108398 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Counterstained with Hematoxylin.



Western blot - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

All lanes : Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398) at 1/2000 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

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 66 kDa **Observed band size:** 68 kDa

Exposure time: 10 seconds

Blocking and diluting buffer: 5% NFDM/TBST



antibody [EPR4097] (ab108398)

Predicted band size: 66 kDa

Exposure time: 180 seconds

Western blot - Anti-Estrogen Receptor alpha

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Lane 1: Recombinant Human Estrogen Receptor beta (aa 1 to 323)

Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398) at

1/1000 dilution + GST-Tagged Recombinant Human Estrogen

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

Receptor beta (aa 1 to 323) protein 100ng

protein, (Cat#: ab158385)

There is no cross-reactivity between ab108398 and ER beta

protein.

Secondary

KDa 1 2

250—
150—
100—
75—
50—
37—
25—
20—
15—
10—

Western blot - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

All lanes: purified at 1/2000 dilution

Lane 1 : MCF-7 cell lysate Lane 2 : T47-D cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

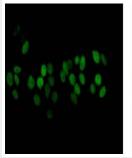
All lanes : Peroxidase-conjugated goat anti-rabbit lgG (H+L) at

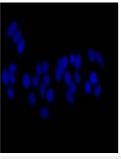
1/1000 dilution

Predicted band size: 66 kDa **Observed band size:** 67 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

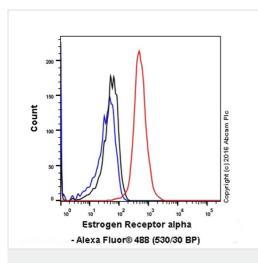
Diluting buffer and concentration: 5% NFDM /TBST.





Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

Immunocytochemsitry/Immunofluorescence analysis of MCF-7 cells labelling Estrogen Receptor alpha (green) with purified ab108398 at 1/200. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).

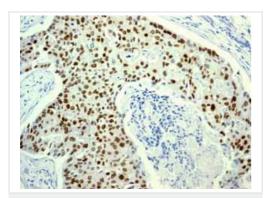


Flow Cytometry (Intracellular) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

ab108398 staining Estrogen Receptor alphain the human cell line MCF-7 (human breast carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilized with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit lgG (Alexa Fluorr® 488) at a dilution of 1/2000 was used as the secondary antibody.

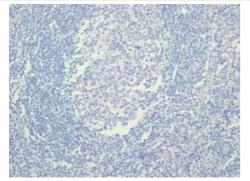
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

Isoytype control: Rabbit monoclonal IgG (Black)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

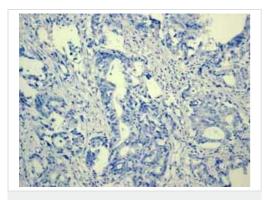
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast ductal infiltrating carcinoma tissue labelling Estrogen Receptor alpha with unpurified ab108398.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha

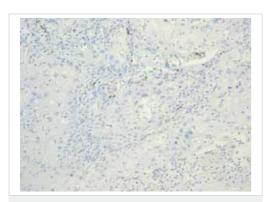
antibody [EPR4097] (ab108398)

Immunohistochemistry (Formalin/PFA-fixed parffin-embedded sections) analysis of human normal tonsil tissue. Unpurified ab108398 shows negative staining.



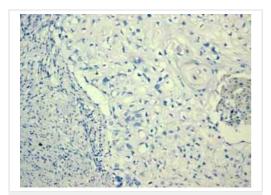
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

Immunohistochemistry (Formalin/PFA-fixed parffin-embedded sections) analysis of human colonic adenocarcinoma tissue.
Unpurified ab108398 shows negative staining.



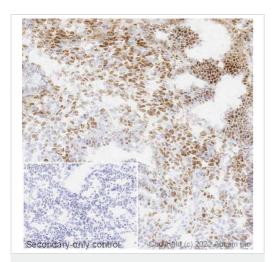
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

Immunohistochemistry (Formalin/PFA-fixed parffin-embedded sections) analysis of human lung adenocarcinoma tissue.
Unpurified ab108398 shows negative staining.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

Immunohistochemistry (Formalin/PFA-fixed parffin-embedded sections) analysis of human cervical carcinoma tissue. Unpurified ab108398 shows negative staining.

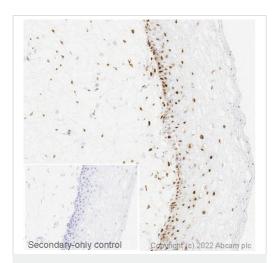


Immunohistochemistry (Frozen sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

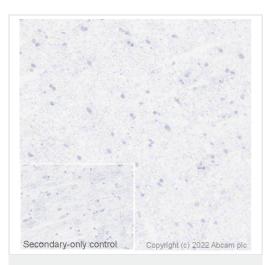
IHC image of Estrogen Receptor alpha staining in a section of frozen human uterus* performed on a Leica Biosystems BOND® RX instrumen using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab108398, 5 ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



Immunohistochemistry (Frozen sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)



Immunohistochemistry (Frozen sections) - Anti-Estrogen Receptor alpha antibody [EPR4097] (ab108398)

IHC image of Estrogen Receptor alpha staining in a section of frozen human cervix* performed on a Leica Biosystems BOND® RX instrumen using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab108398, 5 ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

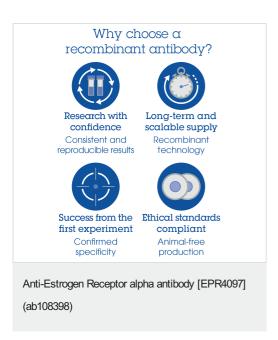
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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Negative control image: IHC image of Estrogen Receptor alpha staining in a section of frozen human hippocampus* performed on a Leica Biosystems BOND® RX instrumen using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab108398, 5 ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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