# abcam

#### Product datasheet

## Anti-Estrogen Receptor alpha antibody [SP1] ab16660

Recombinant RabMAb

★★★★ 7 Abreviews 57 References

16 Images

#### Overview

**Product name** Anti-Estrogen Receptor alpha antibody [SP1]

**Description** Rabbit monoclonal [SP1] to Estrogen Receptor alpha

**Host species** Rahhit

**Tested applications** Suitable for: mIHC, Flow Cyt (Intra), WB, IHC-P, ICC/IF

Species reactivity Reacts with: Human

Predicted to work with: Mouse

Synthetic peptide. This information is considered to be commercially sensitive. **Immunogen** 

**Epitope** C-terminal

Positive control WB: MCF7 cell lysate. IHC-P: Human breast carcinoma, cervix, breast, breast ductal carcinoma

and ovarian adenocarcinoma tissue. ICC/IF: MCF7 cells. Flow Cyt (intra): MCF7 cells. mIHC:

Human triple-positive breast carcinoma, Human mammary gland tissue sections

This product has switched from a hybridoma to recombinant production format on 21st May 2020. **General notes** 

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**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

#### **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: 0.1% Sodium azide Constituents: 1% BSA, PBS

Purity Protein A purified

**Clonality** Monoclonal

Clone number SP1 lsotype lgG

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab16660 in the following tested applications.

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

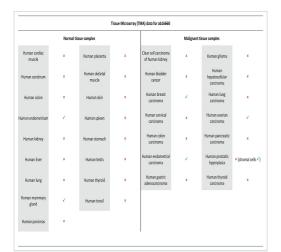
Application	Abreviews	Notes
mIHC		1/200.
Flow Cyt (Intra)		1/200.
WB		1/25. Predicted molecular weight: 67 kDa. Incubate for 1 hour at room temperature.
IHC-P	<b>★★★</b> ☆☆(2)	1/200.
ICC/IF	<b>★★★★★ (1)</b>	1/25 - 1/250.

300		
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**

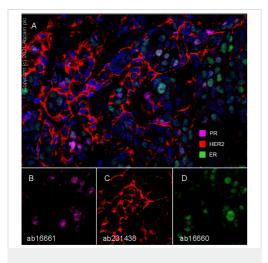
**Target** 



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Tissue Microarrays stained for Anti-Estrogen Receptor alpha antibody [SP1] using ab16660 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested. The section was incubated with ab16660 for 10 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 10 mins.



Multiplex immunohistochemistry - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) analysis of Human triple-positive breast carcinoma tissue sections labeling Estrogen Receptor (ER) with ab16660, at a 1/200 dilution (  $0.07~\mu g/ml$ ). Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins and Opal Polymer HRP Ms + Rb was used as the secondary antibody. DAPI was used as the nuclear counterstain.

Panel A: merged staining of anti-Progesterone Receptor (PR) (magenta; Opal<sup>™</sup>690), anti-HER2 (red; Opal<sup>™</sup>570) and anti-Estrogen Receptor (ER) (green; Opal<sup>™</sup>520) on human triple-positive breast carcinoma.

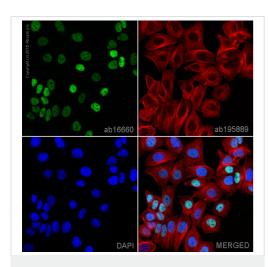
Panel B: anti-PR stained on nucleus of cancer cells.

Panel C: anti-HER2 stained on membrane of cancer cells.

Panel D: anti-ER stained on nucleus of cancer cells.

The section was incubated in three rounds of staining: in the order of <u>ab16661</u> for 30 mins, then ab16660 and <u>ab231438</u> for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

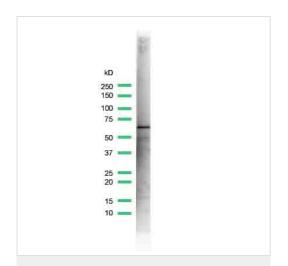
The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.



Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

ab16660 staining Estrogen Receptor alpha in MCF7 cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab16660 at 1/250 dilution (shown in green) and <a href="mailto:ab195889">ab195889</a>, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594), at 2µg/ml (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This image was generated using the hybridoma version of the product.

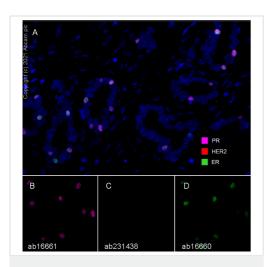


Western blot - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Anti-Estrogen Receptor alpha antibody [SP1] (ab16660) at 1/25 dilution + lysate prepared from MCF7 cells

Predicted band size: 67 kDa

This image was generated using the hybridoma version of the product.



Multiplex immunohistochemistry - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) analysis of Human mammary gland tissue sections labeling Estrogen Receptor (ER) with ab16660, at a 1/200 dilution ( 0.07 µg/ml). Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins and Opal Polymer HRP Ms + Rb was used as the secondary antibody. DAPI was used as the nuclear counterstain.

Panel A: merged staining of anti-Progesterone Receptor (PR) (magenta; Opal™690), anti-HER2 (red; Opal™570) and anti-Estrogen Receptor (ER) (green; Opal™520) on human mammary gland.

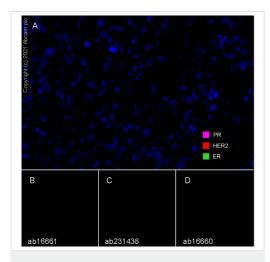
Panel B: anti-PR stained on nucleus of some ductal cells.

Panel C: anti-HER2 stained on no cells.

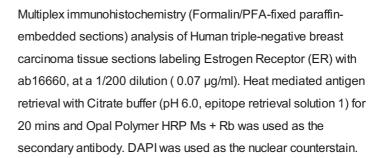
Panel D: anti-ER stained on nucleus of some ductal cells.

The section was incubated in three rounds of staining: in the order of <u>ab16661</u> for 30 mins, then ab16660 and <u>ab231438</u> for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)



Panel A: merged staining of anti-Progesterone Receptor (PR) (magenta; Opal<sup>™</sup>690), anti-HER2 (red; Opal<sup>™</sup>570) and anti-Estrogen Receptor (ER) (green; Opal<sup>™</sup>520) on human triplenegative breast carcinoma.

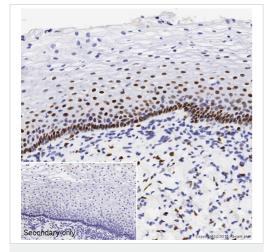
Panel B: anti-PR stained on no cells.

Panel C: anti-HER2 stained on no cells.

Panel D: anti-ER stained on no cells.

The section was incubated in three rounds of staining: in the order of <u>ab16661</u> for 30 mins, then ab16660 and <u>ab231438</u> for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
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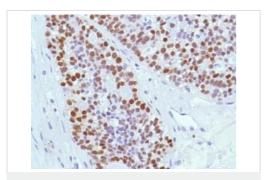
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

IHC image of ab16660 staining Estrogen Receptor alpha in normal human cervix formalin-fixed paraffin-embedded tissue sections\*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab16660, 1/250 dilution, 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. No primary antibody was used in the negative control (shown on the inset).

This image was generated using the hybridoma version of the product.

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.

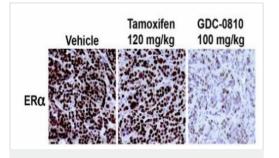
\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Formalin-fixed, paraffin-embedded human ovarian adenocarcinoma tissue stained for Estrogen Receptor alpha using ab16660 at 1/200 dilution in immunohistochemical analysis.

This image was generated using the hybridoma version of the product.

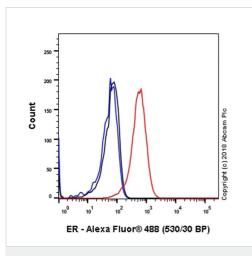


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

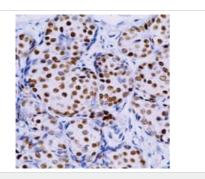
Image courtesy of Joseph J D et al. eLife 2016;5:e15828 doi: 10.7554/eLife.15828

Immunohistochemical analysis of tamoxifen resistant MCF7 xenograft tumours staining estrogen receptor alpha with ab16660. The mice used were treated with a vehicle, tamoxifen (120mg/kg/day p.o.) or GDC-0810 (100mg/kg/day p.o.) for 27 days.

This image was generated using the hybridoma version of the product.



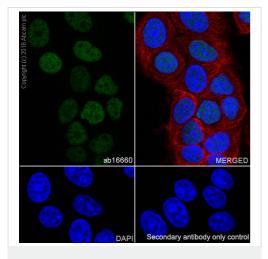
Flow Cytometry (Intracellular) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660) Intracellular flow cytometry analysis of MCF7 (human breast adenocarcinoma epithelial cell) labeling Estrogen Receptor alpha with purified ab16660 at 1/200 dilution (1.06 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal lgG (<u>ab172730</u>) (black). Unlableled control - Unlabelled cells (blue). This image was generated using the hybridoma version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Formalin-fixed, paraffin-embedded human breast carcinoma tissue stained for Estrogen Receptor alpha using ab16660 at 1/200 dilution in immunohistochemical analysis.

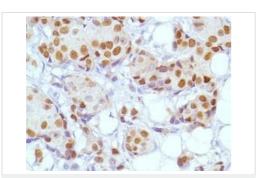
This image was generated using the hybridoma version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (human breast adenocarcinoma epithelial cell) cells labeling Estrogen Receptor alpha with purified ab16660 at 1/25 (8.5  $\mu$ g/ml). Cells were fixed in 100% Methanol. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2  $\mu$ g/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

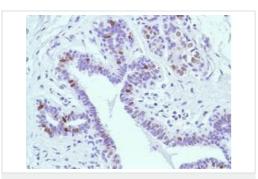
This image was generated using the hybridoma version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Formalin-fixed, paraffin-embedded human breast ductal carcinoma tissue stained for Estrogen Receptor alpha using ab16660 at 1/200 dilution in immunohistochemical analysis.

This image was generated using the hybridoma version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Formalin-fixed, paraffin-embedded human breast tissue stained for Estrogen Receptor alpha using ab16660 at 1/200 dilution in immunohistochemical analysis.

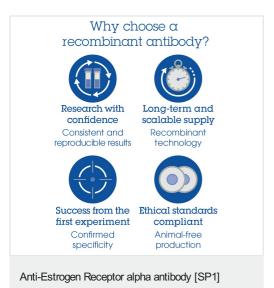
This image was generated using the hybridoma version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

Human breast carcinoma stained with ab16660.

This image was generated using the hybridoma version of the product.



(ab16660)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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