abcam

Product datasheet

Anti-Progesterone Receptor antibody [YR85] ab32085

Recombinant RabMAb

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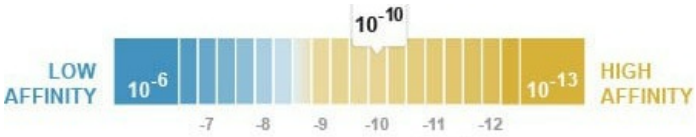
Overview

Product name	Anti-Progesterone Receptor antibody [YR85]
Description	Rabbit monoclonal [YR85] to Progesterone Receptor
Host species	Rabbit
Specificity	This antibody recognises progesterone receptor. The antibody does not cross-react with other NR3 family members. Since the recognized epitope is near the N-terminal end of the protein, this product should only detect isoform B and not isoform A.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Progesterone Receptor (N terminal). The exact sequence is proprietary. Database link: **P06401**
Positive control	Human breast carcinoma, T47D cell lysate. This antibody gave a positive result when used in the following formaldehyde fixed cell lines: DU145.
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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Dissociation constant (K_D)	$K_D = 2.48 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	YR85
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32085 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		1/1000 - 1/10000. Predicted molecular weight: 99 kDa.
IHC-P	★★★★☆ (4)	1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/150.
ICC/IF		Use a concentration of 0.2 - 5 µg/ml.

Target

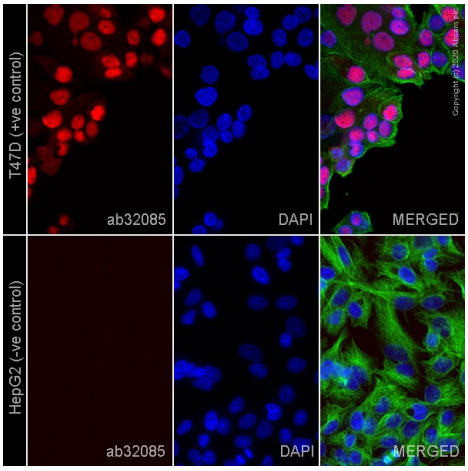
Function	The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues. Progesterone receptor isoform B (PRB) is involved activation of c-SRC/MAPK signaling on hormone stimulation. Isoform A: inactive in stimulating c-Src/MAPK signaling on hormone stimulation. Isoform 4: Increases mitochondrial membrane potential and cellular respiration upon stimulation by progesterone.
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 on multiple serine sites. Several of these sites are hormone-dependent. Phosphorylation on Ser-294 occurs preferentially on isoform B, is highly hormone-dependent and modulates ubiquitination and sumoylation on Lys-388. Phosphorylation on Ser-102 and Ser-345

also requires induction by hormone. Basal phosphorylation on Ser-81, Ser-162, Ser-190 and Ser-400 is increased in response to progesterone and can be phosphorylated in vitro by the CDK2-A1 complex. Increased levels of phosphorylation on Ser-400 also in the presence of EGF, heregulin, IGF, PMA and FBS. Phosphorylation at this site by CDK2 is ligand-independent, and increases nuclear translocation and transcriptional activity. Phosphorylation at Ser-162 and Ser-294, but not at Ser-190, is impaired during the G(2)/M phase of the cell cycle. Phosphorylation on Ser-345 by ERK1/2 MAPK is required for interaction with SP1.
Sumoylation is hormone-dependent and represses transcriptional activity. Sumoylation on all three sites is enhanced by PIAS3. Desumoylated by SENP1. Sumoylation on Lys-388, the main site of sumoylation, is repressed by ubiquitination on the same site, and modulated by phosphorylation at Ser-294.
Ubiquitination is hormone-dependent and represses sumoylation on the same site. Promoted by MAPK-mediated phosphorylation on Ser-294.
Palmitoylated by ZDHHC7 and ZDHHC21. Palmitoylation is required for plasma membrane targeting and for rapid intracellular signaling via ERK and AKT kinases and cAMP generation.

Cellular localization

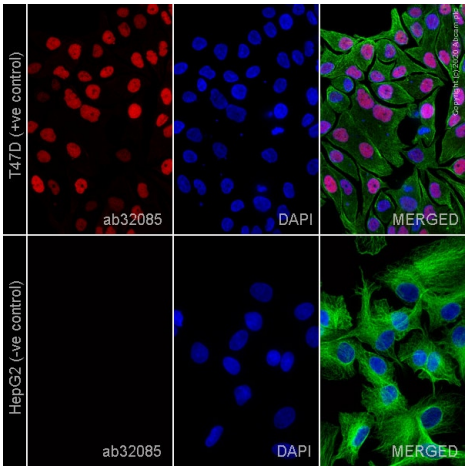
Nucleus. Cytoplasm. Nucleoplasmic shuttling is both homone- and cell cycle-dependent. On hormone stimulation, retained in the cytoplasm in the G(1) and G(2)/M phases; Mitochondrion outer membrane and Nucleus. Cytoplasm. Mainly nuclear.

Images



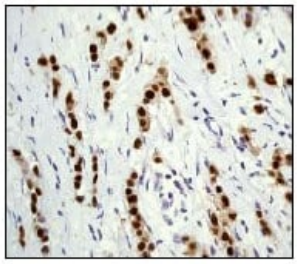
Immunocytochemistry/ Immunofluorescence - Anti-Progesterone Receptor antibody [YR85] (ab32085)

Immunofluorescence staining of Recombinant Anti-Progesterone Receptor antibody [YR85] (ab32085) in T47D cells (+ve control) and HepG2 cells (-ve control). The cells were fixed with 100% Methanol (5 min), 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 ab32085 at 0.2 µg/mL dilution and **ab7291**, Anti-alpha Tubulin antibody [DM1A], at 1/1000 dilution, followed by a further incubation at room temperature for 1h with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) preadsorbed (**ab150087**) at 1/1000 (shown in red) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150117**) at 1/1000 (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with the Perkin Elmer Operetta and a maximum intensity projection of confocal planes is shown.



Immunocytochemistry/ Immunofluorescence - Anti-Progesterone Receptor antibody [YR85] (ab32085)

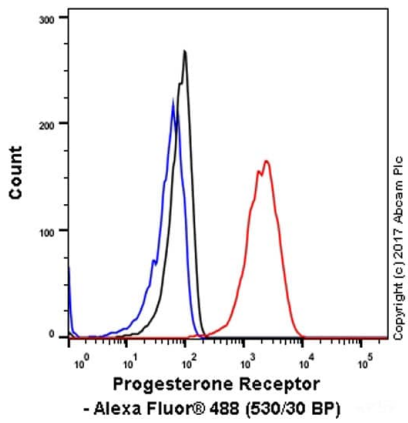
Immunofluorescence staining of Recombinant Anti-Progesterone Receptor antibody [YR85] (ab32085) in T47D cells (+ve control) and HepG2 cells (-ve control). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab32085 at 0.2 µg/mL dilution and **ab7291**, Anti-alpha Tubulin antibody [DM1A], at 1/1000 dilution, followed by a further incubation at room temperature for 1h with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) preadsorbed (**ab150087**) at 1/1000 (shown in red) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150117**) at 1/1000 (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with the Perkin Elmer Operetta and a maximum intensity projection of confocal planes is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Progesterone Receptor antibody [YR85] (ab32085)

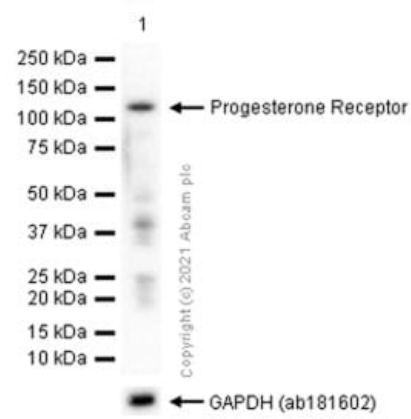
Immunohistochemical analysis of progesterone receptor expression in paraffin embedded human breast carcinoma, using 1/100 ab32085.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Progesterone Receptor antibody [YR85] (ab32085)

Intracellular Flow Cytometry analysis of T47D (human mammary gland ductal carcinoma) cells labeling Progesterone Receptor with purified ab32085 at 1/1100 dilution (1ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) (**ab172730**) was used as the isotype control, Cell without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-Progesterone Receptor antibody [YR85] (ab32085)

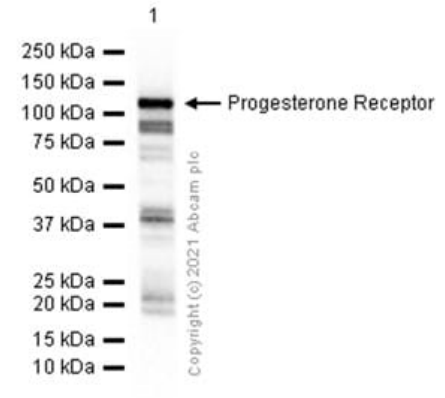
Anti-Progesterone Receptor antibody [YR85] (ab32085) at 1/1000 dilution + Human breast cancer lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/20000 dilution

Predicted band size: 99 kDa
Observed band size: 118 kDa

Exposure time: 180 seconds



Western blot - Anti-Progesterone Receptor antibody [YR85] (ab32085)

Anti-Progesterone Receptor antibody [YR85] (ab32085) at 1/2000 dilution + T-47D (Human ductal breast epithelial tumor epithelial cell) whole cell lysate at 15 µg

Secondary

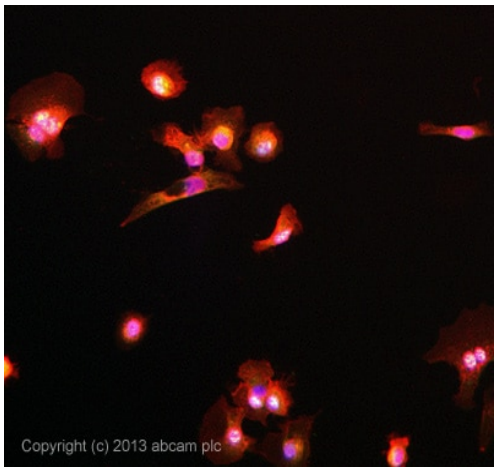
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 99 kDa

Observed band size: 118 kDa

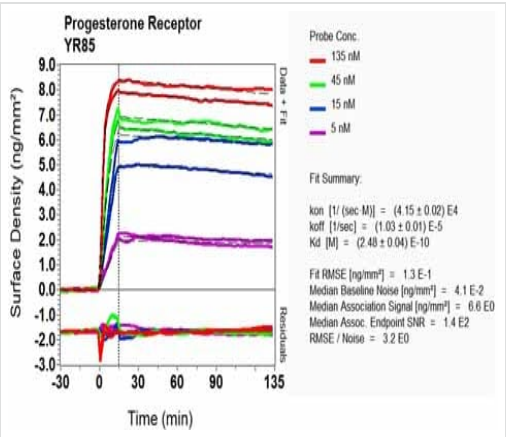
Exposure time: 40 seconds

Blocking buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Progesterone Receptor antibody [YR85] (ab32085)

ICC/IF image of ab32085 stained DU145 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab32085 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (**ab96899**) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



OI-RD Scanning - Anti-Progesterone Receptor antibody [YR85] (ab32085)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-Progesterone Receptor antibody [YR85] (ab32085)

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