abcam

Product datasheet

Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] ab275745

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271]
Description	Rabbit monoclonal [EPR23871-271] to Retinoic Acid Receptor alpha
Host species	Rabbit
Tested applications	**Suitable for:** IP, WB, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human testis tissue lysate; HeLa, Hep G2, NIH/3T3, PC-12, MCF7, U937 and HEK-293 whole cell lysates. IHC-P: Human testis tissue; Mouse testis tissue; Rat testis tissue. IP: HEK-293 and NIH/3T3 whole cell lysates.
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**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR23871-271
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275745 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
IP		1/30.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 52 kDa (predicted molecular weight: 51 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Receptor for retinoic acid. Retinoic acid receptors bind as heterodimers to their target response elements in response to their ligands, all-trans or 9-cis retinoic acid, and regulate gene expression in various biological processes. The RXR/RAR heterodimers bind to the retinoic acid response elements (RARE) composed of tandem 5'-AGGTCA-3' sites known as DR1-DR5. In the absence of ligand, the RXR-RAR heterodimers associate with a multiprotein complex containing transcription corepressors that induce histone acetylation, chromatin condensation and transcriptional suppression. On ligand binding, the corepressors dissociate from the receptors and associate with the coactivators leading to transcriptional activation. RARA plays an essential role in the regulation of retinoic acid-induced germ cell development during spermatogenesis. Has a role in the survival of early spermatocytes at the beginning prophase of meiosis. In Sertoli cells, may promote the survival and development of early meiotic prophase spermatocytes. In concert with RARG, required for skeletal growth, matrix homeostasis and growth plate function (By similarity). Regulates expression of target genes in a ligand-dependent manner by recruiting chromatin complexes containing MLL5. Mediates retinoic acid-induced granulopoiesis.

Involvement in disease Note=Chromosomal aberrations involving RARA are commonly found in acute promyelocytic leukemia. Translocation t(11;17)(q32;q21) with ZBTB16/PLZF; translocation t(15;17)(q21;q21) with PML; translocation t(5;17)(q32;q11) with NPM. The PML-RARA oncoprotein requires both the PML ring structure and coiled-coil domain for both interaction with UBE2I, nuclear microspeckle location and sumoylation. In addition, the coiled-coil domain functions in blocking RA-mediated transactivation and cell differentiation.

Sequence similarities Belongs to the nuclear hormone receptor family. NR1 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.

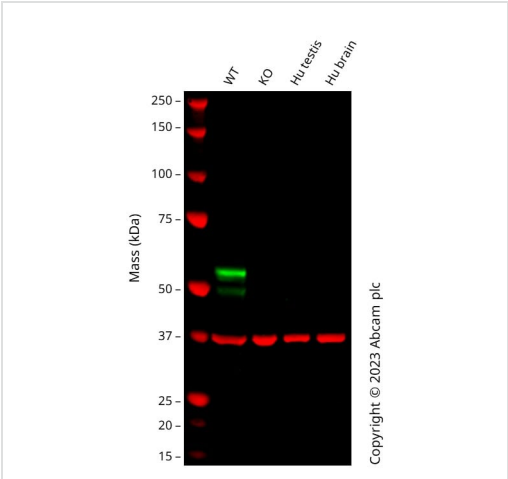
Post-translational modifications Phosphorylated on serine and threonine residues. Phosphorylation does not change during cell cycle. Phosphorylation on Ser-77 is crucial for transcriptional activity (By similarity). Phosphorylation by AKT1 is required for the repressor activity but has no effect on DNA binding,

protein stability nor subcellular localization. Phosporylated by PKA in vitro. This phosphorylation on Ser-219 and Ser-369 is critical for ligand binding, nuclear localization and transcriptional activity in response to FSH signaling.
Sumoylated by SUMO2, mainly on Lys-399 which is also required for SENP6 binding. On all-trans retinoic acid (ATRA) binding, a confromational change may occur that allows sumoylation on two additional site, Lys-166 and Lys-171. Probably desumoylated by SENP6. Sumoylation levels determine nuclear localization and regulate ATRA-mediated transcriptional activity.
Trimethylation enhances heterodimerization with RXRA and positively modulates the transcriptional activation.
Ubiquitinated.

Cellular localization

Nucleus. Cytoplasm. Nuclear localization depends on ligand binding, phosphorylation and sumoylation. Transloaction to the nucleus in the absence of ligand is dependent on activation of PKC and the downstream MAPK phosphorylation.

Images



Western blot - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

All lanes : Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745) at 1/1000 dilution

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : RARA knockout MCF7 cell lysate
Lane 3 : Human Testis cell lysate
Lane 4 : Human Brain cell lysate

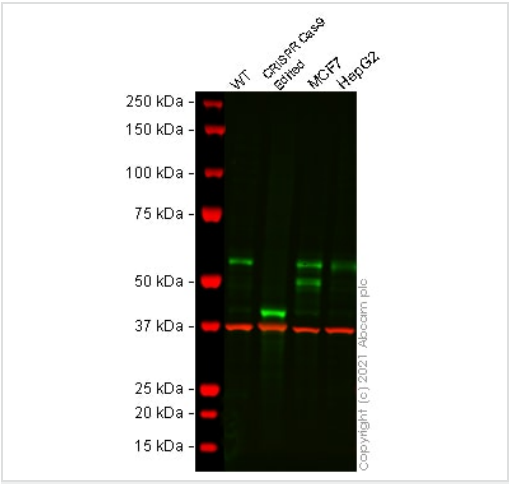
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 51 kDa
Observed band size: 50-55 kDa

Anti-RARA antibody [EPR23871-271] (ab275745) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab275745 was shown to bind specifically to RARA. A band was observed at 50-55 kDa in wild-type MCF7 cell lysates with no signal observed at this size in RARA knockout cell line **ab277158** (knockout cell lysate **ab281383**). To generate this image, wild-type and RARA knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were

washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

All lanes : Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RARA CRISPR-Cas9 edited HeLa cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : HepG2 cell lysate

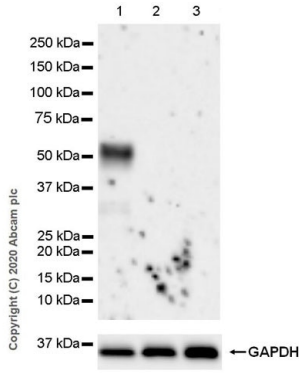
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 51 kDa
Observed band size: 40 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab275745 observed at 40 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab275745 was shown to react with Retinoic Acid Receptor alpha in wild-type HeLa cells in western blot. The bands observed in RARA CRISPR/Cas9 edited cell line **ab265176** (RARA CRISPR/Cas9 edited cell lysate **ab257629**) below 40 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and RARA CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab275745 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

All lanes : Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745) at 1/1000 dilution

Lane 1 : Human testis tissue lysate
Lane 2 : Human brain tissue lysate
Lane 3 : Human skeletal muscle tissue lysate

Lysates/proteins at 20 µg per lane.

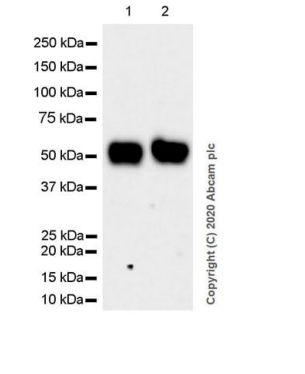
Secondary
All lanes : VeriBlot for IP secondary antibody(HRP)(**ab131366**) at 1/1000 dilution

Predicted band size: 51 kDa
Observed band size: 52 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Low expression: human brain, human skeletal muscle (Database:GTExPortal, HPA).

Exposure time: 103 seconds.



Western blot - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

All lanes : Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745) at 1/1000 dilution

Lane 1 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 2 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

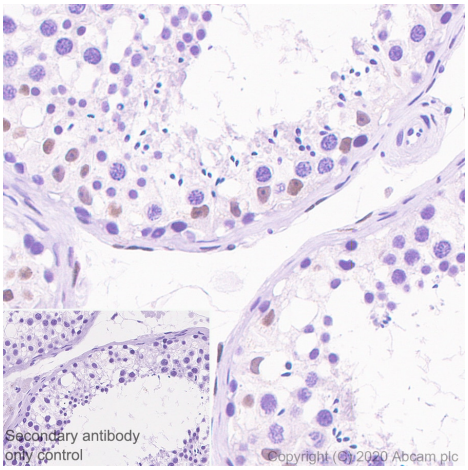
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/20000 dilution

Predicted band size: 51 kDa
Observed band size: 52 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 70 seconds.

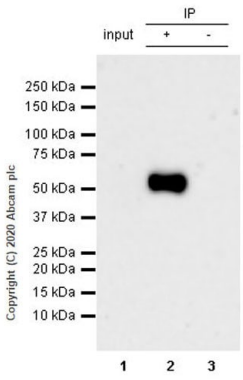


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling Retinoic Acid Receptor alpha with ab275745 at 1/1000 (2.71 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Nuclear staining on sertoli cells of human testis. The section was incubated with ab275745 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunoprecipitation - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

Retinoic Acid Receptor alpha was immunoprecipitated from 0.35 mg HEK-293 (human embryonic kidney epithelial cell) whole cell lysate with ab275745 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab275745 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

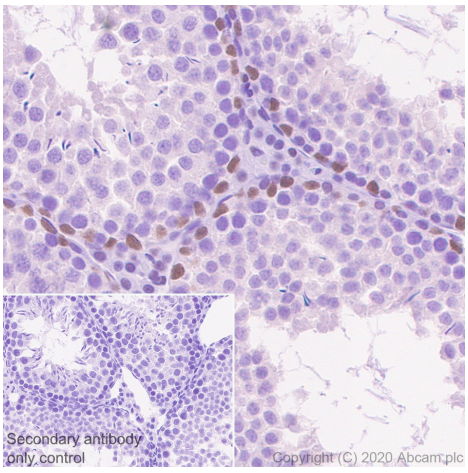
Lane 1: HEK-293 (human embryonic kidney epithelial cell) whole cell lysate 10 ug

Lane 2: ab275745 IP in HEK-293 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab275745 in HEK-293 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 84 seconds.

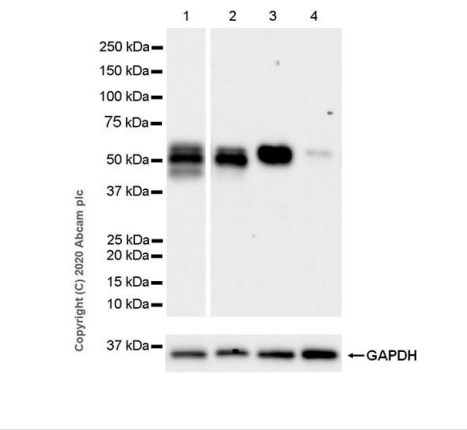


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling Retinoic Acid Receptor alpha with ab275745 at 1/1000 (0.542 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Nuclear staining on sertoli cells of rat testis. The section was incubated with ab275745 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745)

All lanes : Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (ab275745) at 1/1000 dilution

Lane 1 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : U937 (human histiocytic lymphoma monocyte) whole cell lysate
Lane 3 : HEK-293 (human embryonic kidney epithelial cell) whole cell lysate
Lane 4 : MOLT-4 (human lymphoblastic leukemia T lymphoblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

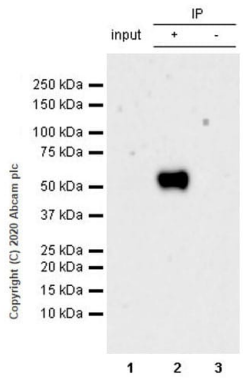
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/20000 dilution

Predicted band size: 51 kDa
Observed band size: 52 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Low expression: MOLT-4.(Database:HPA).

Exposure time: Lane 1: 8 seconds Lane2-4: 70 seconds.



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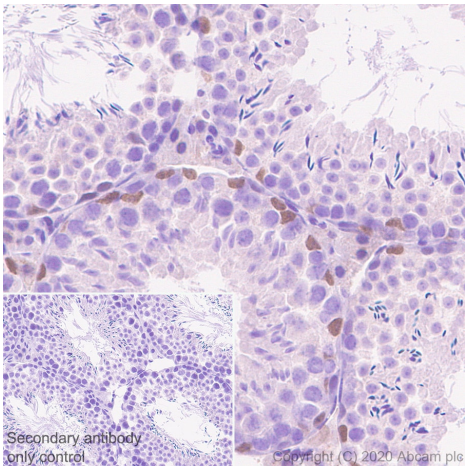
Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 ug

Lane 2: ab275745 IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab275745 in NIH/3T3 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 84 seconds.



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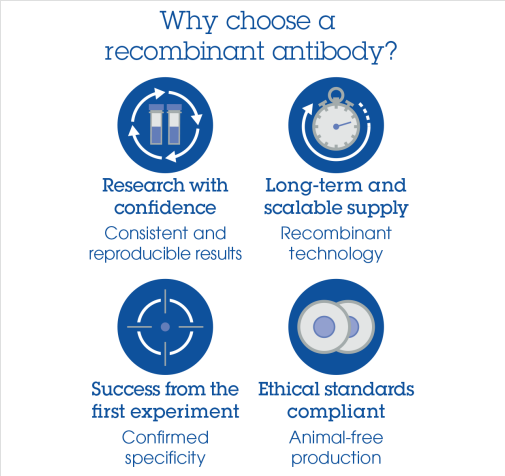
Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling Retinoic Acid Receptor alpha with ab275745 at 1/1000 (0.542 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Nuclear staining on sertoli cells of mouse testis (PMID:16210368). The section was incubated with ab275745 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Anti-Retinoic Acid Receptor alpha antibody

[EPR23871-271] (ab275745)

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

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