abcam

Product datasheet

Anti-CYP11A1 antibody [EPR24868-86] ab272494

Recombinant RabMAb

16 Images

Overview

Product name	Anti-CYP11A1 antibody [EPR24868-86]
Description	Rabbit monoclonal [EPR24868-86] to CYP11A1
Host species	Rabbit
Specificity	ICC application does not react with Mouse and Rat species. Flow Cyt application does not react with Mouse species.
Tested applications	**Suitable for:** WB, mIHC, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human adrenal gland, Human testis, JEG-3, Mouse testis, Rat testis, Mouse ovary, Rat ovary, NIH/3T3, PC-12 and Rat-1 lysates. IHC-P: Human adrenal gland, Human testis, Mouse testis, Rat adrenal gland tissues. ICC/IF: JEG-3 cells. Flow Cyt: JEG-3 and PC-12 cells. IP: JEG-3, Mouse testis and Rat testis cells. mIHC: Human testis.
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR24868-86
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab272494 in the following tested applications.

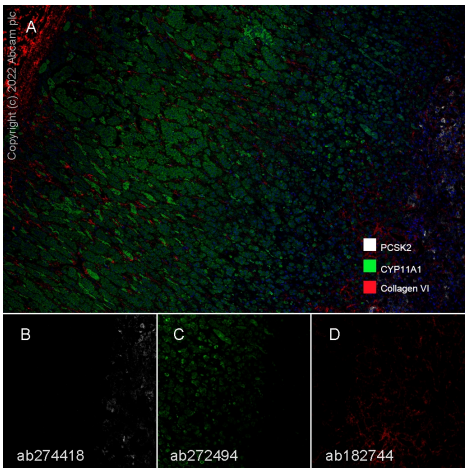
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Predicted molecular weight: 60 kDa.
mIHC		1/10000.
IHC-P		1/10000.
ICC/IF		1/50. ICC application does not react with Mouse and Rat species.
IP		1/30.
Flow Cyt (Intra)		1/50. FC application does not react with Mouse species.

Target

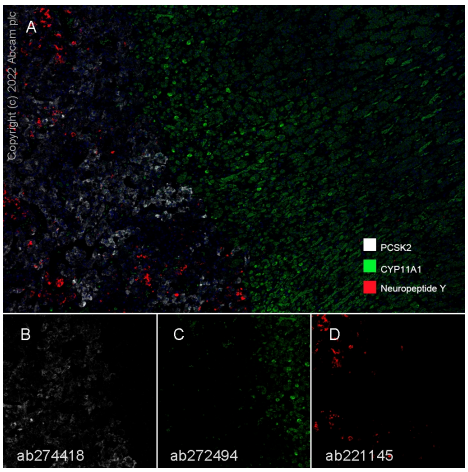
Function	Catalyzes the side-chain cleavage reaction of cholesterol to pregnenolone.
Pathway	Lipid metabolism; C21-steroid hormone metabolism.
Involvement in disease	Defects in CYP11A1 are a cause of congenital adrenal insufficiency (CAI). Defects in CYP11A1 are a cause of congenital lipoid adrenal hyperplasia (CLAH) [MIM:201710]; also known as lipoid CAH. CLAH is the most severe form of adrenal hyperplasia. This autosomal recessive and potentially lethal condition includes the onset of profound adrenocortical insufficiency shortly after birth, hyperpigmentation reflecting increased production of pro-opiomelanocortin, elevated plasma renin activity as a consequence of reduced aldosterone synthesis, and male pseudohermaphroditism resulting from deficient fetal testicular testosterone synthesis. CLAH is a rare disease, except in Japan and Korea where it accounts for a significant percentage of cases of congenital adrenal hyperplasia.
Sequence similarities	Belongs to the cytochrome P450 family.
Cellular localization	Mitochondrion membrane.

Images



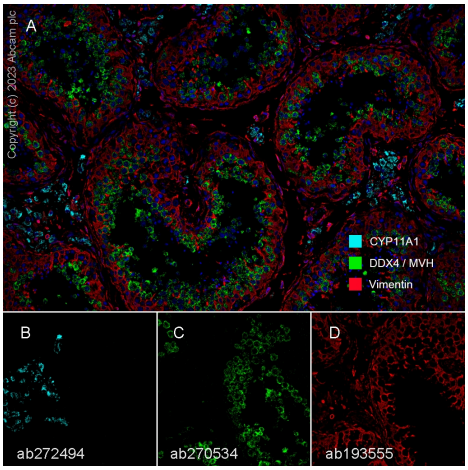
Multiplex immunohistochemistry - Anti-CYP11A1 antibody [EPR24868-86] (ab272494)

Fluorescence multiplex immunohistochemical analysis of human adrenal gland (formalin-fixed paraffin-embedded section). Panel A shows merged staining of anti-PCSK2 stained on adrenal medulla (**ab274418**; gray; Opal™690) at 1:2000 (0.263 µg/ml) [Panel B], anti-CYP11A1 stained on adrenal cortex (ab272494; green; Opal™520) at 1:10000 (0.053 µg/ml) [Panel C], and anti-Collagen VI stained on extracellular matrix (**ab182744**; red; Opal™570) at 1:1000 (1.518 µg/ml) [Panel D] on human adrenal gland. DAPI was used as a nuclear counter stain. Followed by Opal Polymer HRP Ms + Rb secondary antibody. 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. The section was incubated in three rounds of staining: in the order of **ab274418**, ab272494, and **ab182744** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used.



Multiplex immunohistochemistry - Anti-CYP11A1 antibody [EPR24868-86] (ab272494)

Fluorescence multiplex immunohistochemical analysis of human adrenal gland (formalin-fixed paraffin-embedded section). Panel A shows merged staining of anti-PCSK2 stained on adrenal medulla (**ab274418**; gray; Opal™690) at 1:2000 (0.263 µg/ml) [Panel B], anti-CYP11A1 stained on adrenal cortex (ab272494; green; Opal™520) at 1:10000 (0.053 µg/ml) [Panel C], and anti-Neuropeptide Y stained on chromaffin cells (**ab221145**; red; Opal™570) at 1:2000 (0.279 µg/ml) [Panel D] on human adrenal gland. DAPI was used as a nuclear counter stain. Followed by Opal Polymer HRP Ms + Rb secondary antibody. 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. The section was incubated in three rounds of staining: in the order of **ab274418**, ab272494, and **ab221145** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used.



Multiplex immunohistochemistry - Anti-CYP11A1 antibody [EPR24868-86] (ab272494)

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue.

Panel A: Merged staining of anti-Vimentin (**ab193555**; red; Opal™690), anti-CYP11A1 (ab272494; cyan; Opal™520) and anti-DDX4 / MVH (**ab270534**; green; Opal™570) on human testis.

Panel B: Anti-CYP11A1 stained on Leydig cells.

Panel C: Anti-DDX4 / MVH stained on all spermatogenic cell types.

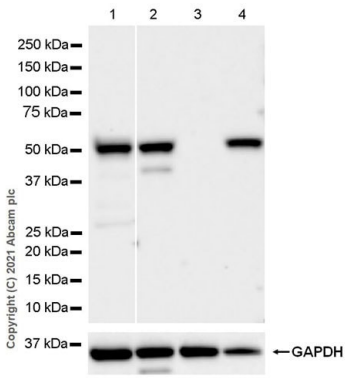
Panel D: Anti-Vimentin stained on Sertoli cells and fibroblasts.

Key protocol steps: The section was incubated in three rounds of staining: in the order of **ab193555** (1:2000 dilution) and ab272494 (1:10000 dilution) for 30 mins, then **ab270534** (1:2000 dlilution) 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.

DAPI was used as a nuclear counter stain. Opal Polymer HRP Ms + Rb was used as a secondary.

Antigen retrieval: Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-CYP11A1 antibody [EPR24868-86] (ab272494)

All lanes : Anti-CYP11A1 antibody [EPR24868-86] (ab272494) at 1/1000 dilution

Lane 1 : Human adrenal gland tissue lysate
Lane 2 : Human testis tissue lysate
Lane 3 : Human heart tissue lysate
Lane 4 : JEG-3 (Human placenta epithelial choriocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

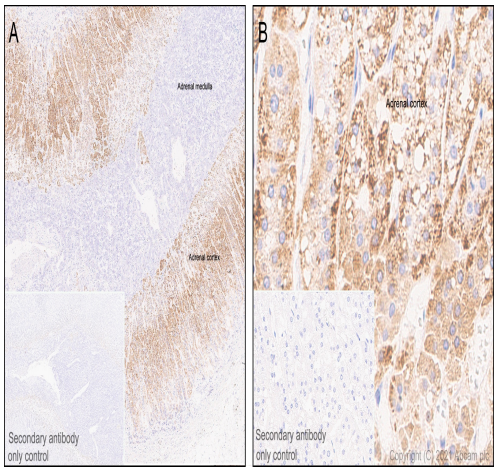
Predicted band size: 60 kDa
Observed band size: 50 kDa

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

Negative control: human heart (PMID:19491374, 21520051)

The band below (~37 kDa) in lane 2 may be caused by degradation.

Exposure time: Lane 1: 7.75 seconds Lane 2-4: 37 seconds

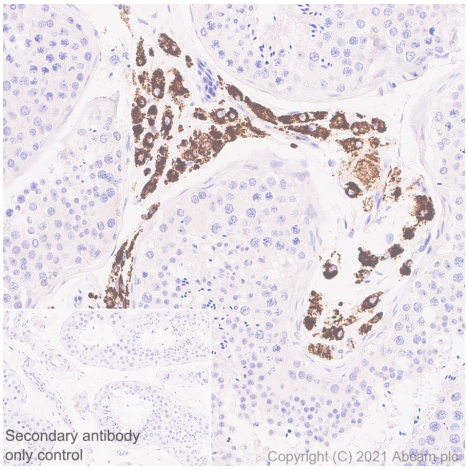


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CYP11A1 antibody (ab272494)

Immunohistochemical analysis of paraffin-embedded Human adrenal gland tissue labelling CYP11A1 with ab272494 at 1/10000 (0.054 ug/ml) followed by a ready to use LeicaDS9800 (BNOD™, Polymer Refine Detection) was used. Positive staining on human adrenal cortex (PMID: 22796438). (A) Low-powered (magnification, x40) and (B) high-powered (magnification, x400) microscopic images. The section was incubated with ab272494 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 with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 minsn was used.

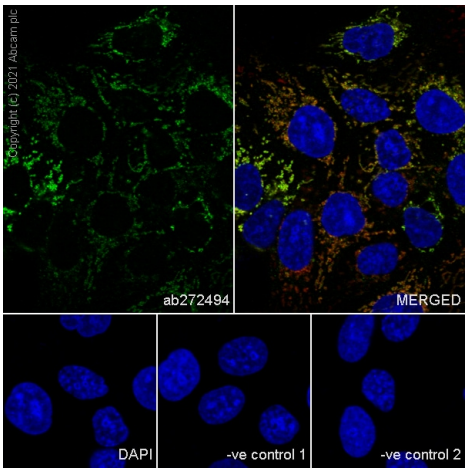


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CYP11A1 antibody (ab272494)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labelling CYP11A1 with ab272494 at 1/10000 (0.054 ug/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on Leydig cells of human testis. The section was incubated with ab272494 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.

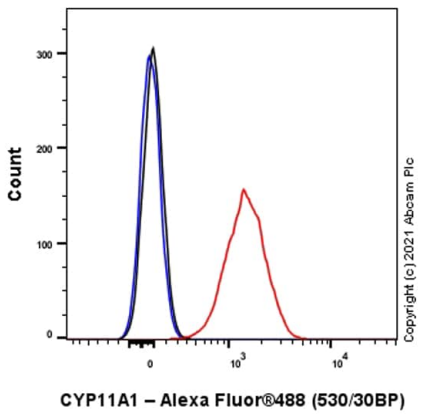
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-CYP11A1 antibody [EPR24868-86] (ab272494)

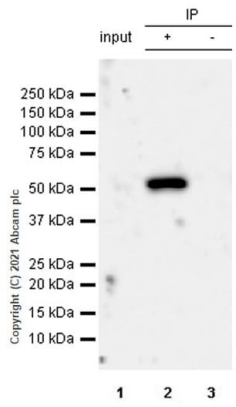
Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized JEG-3 (human placenta epithelial choriocarcinoma) cells labelling CYP11A1 with ab272494 at 1/50 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining colocalized with mitochondrial marker (**ab33985**) in JEG-3 cell line is observed. **ab33985** Anti-COX IV mouse monoclonal antibody - Mitochondrial Marker was used to counterstain mitochondrion at 1/1000 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-CYP11A1 antibody (ab272494)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized JEG-3 (Human placenta epithelial choriocarcinoma) cells labelling CYP11A1 with ab272494 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CYP11A1 antibody (ab272494)

CYP11A1 was immunoprecipitated from 0.35 mg JEG-3 (Human placenta epithelial choriocarcinoma) whole cell lysate with ab272494 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab272494 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

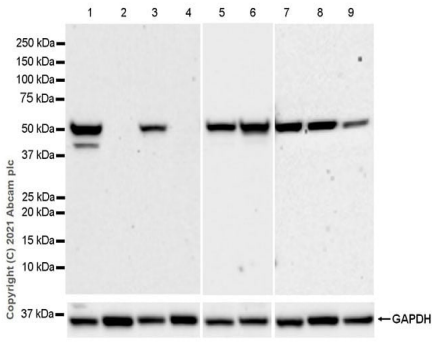
Lane 1: JEG-3 (Human placenta epithelial choriocarcinoma) whole cell lysate 10µg

Lane 2: ab272494 IP in JEG-3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab272494 in JEG-3 whole cell lysate

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

Exposure time: 58 seconds



Western blot - Anti-CYP11A1 antibody [EPR24868-86] (ab272494)

All lanes : Anti-CYP11A1 antibody [EPR24868-86] (ab272494) at 1/1000 dilution

Lane 1 : Mouse testis tissue lysate
Lane 2 : Mouse brain tissue lysate
Lane 3 : Rat testis tissue lysate
Lane 4 : Rat brain tissue lysate
Lane 5 : Mouse ovary tissue lysate
Lane 6 : Rat ovary tissue lysate
Lane 7 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 8 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 9 : Rat-1 (rat embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 60 kDa
Observed band size: 50 kDa

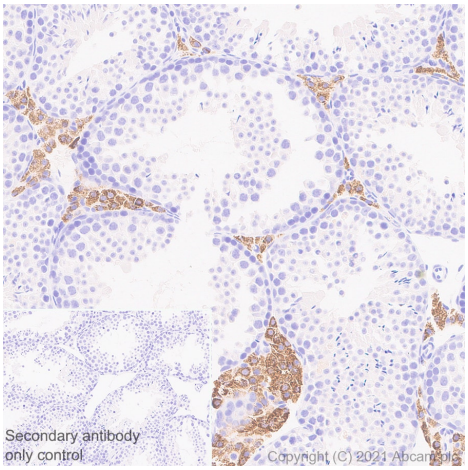
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Low expression: brain (PMID: 21520051, 19491374)

Lane 7-9: This blot was developed using a higher sensitivity ECL substrate.

The band below (~37 kDa) in lane 1 may be caused by degradation.

Exposure time: Lane 1-4, 7-9: 3 minutes Lane 5-6: 37 seconds

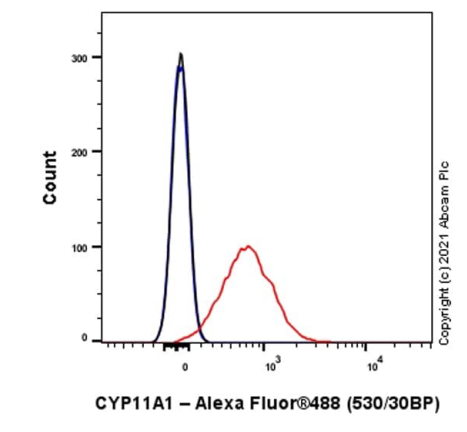


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CYP11A1 antibody (ab272494)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labelling CYP11A1 with ab272494 at 1/10000 (0.054 ug/ml) followed by a ready to use LeicaDS9800(BOND™ Polymer Refine Detection) was used. Positive staining on Leydig cells of mouse testis. The section was incubated with ab272494 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

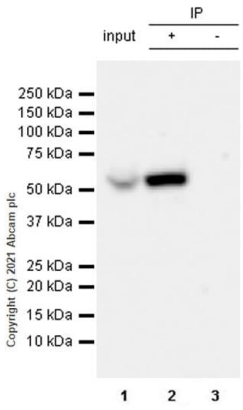
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-CYP11A1 antibody (ab272494)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized PC-12 (Rat adrenal gland pheochromocytoma) cells labelling CYP11A1 with ab272494 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CYP11A1 antibody (ab272494)

CYP11A1 was immunoprecipitated from 0.35 mg Mouse testis tissue lysate with ab272494 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab272494 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

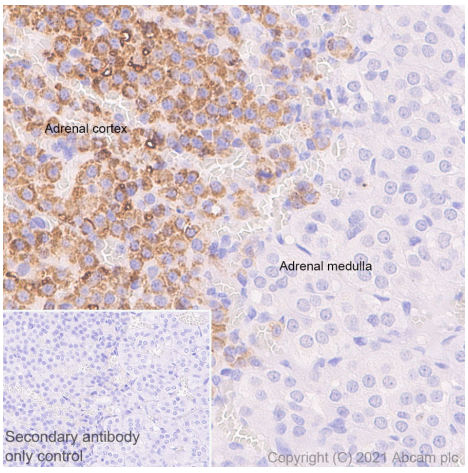
Lane 1: Mouse testis tissue lysate 10µg

Lane 2: ab272494 IP in Mouse testis tissue lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab272494 in mouse testis tissue lysate

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

Exposure time: 10 seconds

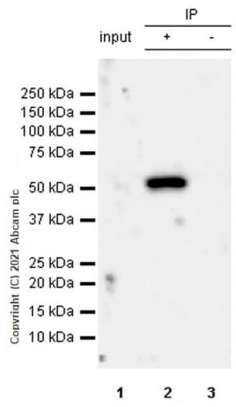


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CYP11A1 antibody (ab272494)

Immunohistochemical analysis of paraffin-embedded Rat adrenal gland tissue labelling CYP11A1 with ab272494 at 1/10000 (0.054 ug/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection)was used. Positive staining on rat adrenal cortex. The section was incubated with ab272494 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 with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunoprecipitation - Anti-CYP11A1 antibody (ab272494)

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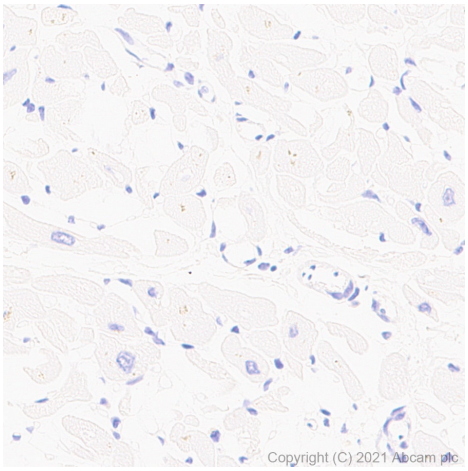
Lane 1: Rat testis tissue lysate 10µg

Lane 2: ab272494 IP in Rat testis tissue lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab272494 in rat testis tissue lysate

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

Exposure time: 58 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CYP11A1 antibody (ab272494)

Immunohistochemical analysis of paraffin-embedded Human cardiac muscle tissue labelling CYP11A1 with ab272494 at 1/10000 (0.054 ug/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Negative control: no staining on human cardiac muscle. The section was incubated with ab272494 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 with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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