## abcam

#### Product datasheet

### Anti-POMC antibody [EPR22534-165] ab254257



★★★★★ 1 Abreviews 1 References

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#### Overview

**Product name** Anti-POMC antibody [EPR22534-165]

**Description** Rabbit monoclonal [EPR22534-165] to POMC

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-P, IHC-Fr, ICC/IF

Unsuitable for: IP

Species reactivity Reacts with: Mouse. Rat. Human

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

Positive control WB: Mouse, human and rat pituitary tissue lysate. IHC-P: Mouse pituitary and spleen; human and

rat pituitary tissue. IHC-Fr: Mouse and rat pituitary and liver tissue. ICC/IF: AtT-20 cells.

**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  $\mathsf{RabMAb}^{\texttt{®}}$  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 pH: 7.2

Preservative: 0.01% Sodium azide

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

**Purity** Protein A purified

Clonality Monoclonal

Clone number EPR22534-165

#### **Applications**

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab254257 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. Detects a band of approximately 35 kDa (predicted molecular weight: 29 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr	<b>★★★★</b> <u>(1)</u>	1/500.
ICC/IF		1/100.

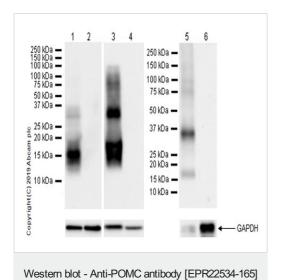
**Application notes** 

Is unsuitable for IP.

#### **Target**

#### **Images**

(ab254257)



**Lanes 1-4**: Anti-POMC antibody [EPR22534-165] (ab254257) at 1/5000 dilution

**Lanes 5-6**: Anti-POMC antibody [EPR22534-165] (ab254257) at 1/1000 dilution

Lane 1: Mouse pituitary lysate

Lane 2: Mouse liver lysate

Lane 3: Human pituitary lysate

Lane 4: Human liver lysate

Lane 5: Rat pituitary lysate

Lane 6: Rat liver lysate

Lysates/proteins at 20  $\mu g$  per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 29 kDa

Observed band size: 35, 16 kDa

Exposure time:

Lanes 1-2: 3 seconds;

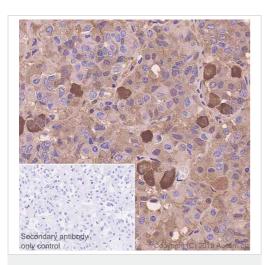
Lanes 3-4: 10 seconds;

Lanes 5-6: 32 seconds

Blocking buffer and concentration: 5% NFDM/TBST

Negative control: liver (lanes 2, 4 and 6, PMID: 10395956)

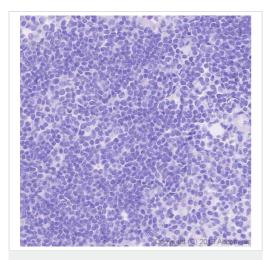
POMC is a glycoprotein and can be processed into 16kDa N-POMC. The molecular weight observed is consistent with what has been described in the literature (PMID 1814247, PMID 26880796, PMID 20639461).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-POMC antibody
[EPR22534-165] (ab254257)

Immunohistochemical analysis of paraffin-embedded human pituitary tissue labeling POMC with ab254257 at 1/4000 dilution at room temperature for 30 mins, followed by ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human pituitary is observed (PMID: 21622576). Counterstained with hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was performed. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

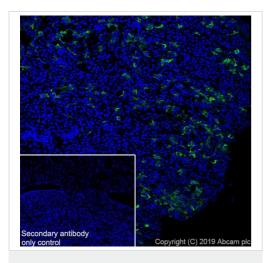


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-POMC antibody

[EPR22534-165] (ab254257)

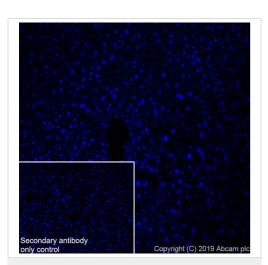
# Negative control: No staining on mouse spleen (PMID: 10395956)

Immunohistochemical analysis of paraffin-embedded mouse pituitary tissue labeling POMC with ab254257 at 1/4000 dilution at room temperature for 30 mins, followed by ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counterstained with hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was performed. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Frozen sections) - Anti-POMC antibody [EPR22534-165] (ab254257)

Immunohistochemical analysis of 0.2% Triton X-100 permeabilized, 4% PFA fixed mouse pituitary tissue labeling POMC with ab254257 at 1/500 dilution, followed by AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution. DAPI was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed. Positive staining on mouse pituitary (PMID: 18601691). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit



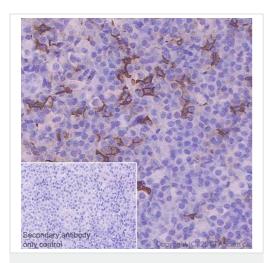
Immunohistochemistry (Frozen sections) - Anti-POMC antibody [EPR22534-165] (ab254257)

#### Negative control: No stain on liver (PMID: 14578285)

secondary (ab150077) at 1/1000 dilution

Immunohistochemical analysis of 0.2% Triton X-100 permeabilized, 4% PFA fixed mouse liver tissue labeling POMC with ab254257 at 1/100 dilution, followed by AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution. DAPI was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed. No staining on mouse liver.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution

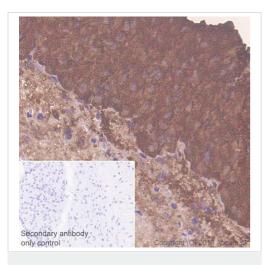


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-POMC antibody

[EPR22534-165] (ab254257)

Immunohistochemical analysis of paraffin-embedded rat pituitary tissue labeling POMC with ab254257 at 1/4000 dilution at room temperature for 30 mins, followed by ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat pituitary is observed (PMID: 21622576). Counterstained with hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was performed. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

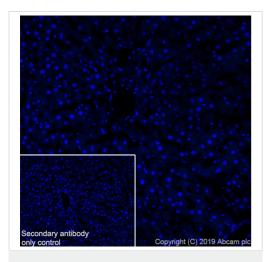
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-POMC antibody
[EPR22534-165] (ab254257)

Immunohistochemical analysis of paraffin-embedded mouse pituitary tissue labeling POMC with ab254257 at 1/4000 dilution at room temperature for 30 mins, followed by ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse pituitary is observed (PMID: 21622576). Counterstained with hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was performed. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

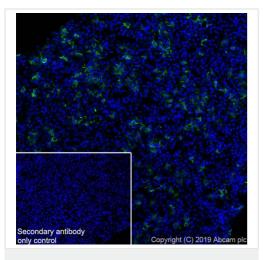


Immunohistochemistry (Frozen sections) - Anti-POMC antibody [EPR22534-165] (ab254257)

#### Negative control: No stain on liver (PMID: 14578285)

Immunohistochemical analysis of 0.2% Triton X-100 permeabilized, 4% PFA fixed rat liver tissue labeling POMC with ab254257 at 1/100 dilution, followed by AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution. DAPI was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed. No staining on rat liver.

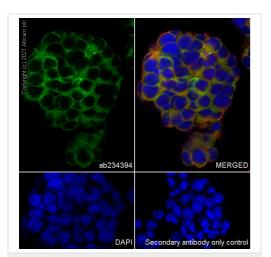
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution



Immunohistochemistry (Frozen sections) - Anti-POMC antibody [EPR22534-165] (ab254257)

Immunohistochemical analysis of 0.2% Triton X-100 permeabilized, 4% PFA fixed rat pituitary tissue labeling POMC with ab254257 at 1/500 dilution, followed by AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution. DAPI was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed. Positive staining on rat pituitary (PMID: 18601691).

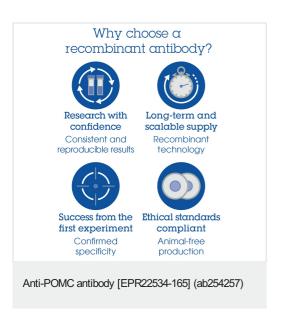
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (ab150077) at 1/1000 dilution



Immunocytochemistry/ Immunofluorescence - Anti-POMC antibody [EPR22534-165] (ab254257)

Immunocytochemical analysis of 100% methanol fixed, 0.1% TritonX-100 permeabilised AtT-20 cells labeling POMC with ab254257 at 1/100 dilution. <a href="mailto:ab150077">ab150077</a> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as a secondary antibody at 1/1000 dilution. Counterstained with <a href="mailto:ab195889">ab195889</a> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution. Nuclear staining: DAPI.

Confocal image showing cytoplasmic staining in AtT-20 cell line



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