# abcam

### Product datasheet

## Anti-GRP94 antibody [EPR22847-50] ab238126



#### 15 References 画像数 11

#### 製品の概要

製品名 Anti-GRP94 antibody [EPR22847-50]

製品の詳細 Rabbit monoclonal [EPR22847-50] to GRP94

由来種 Rabbit

アプリケーション 適用あり: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)

種交差性 交差種: Mouse. Rat. Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Human kidney and stomach tissue lysates; mouse brain and placenta tissue lysates; rat

> brain, placenta and stomach tissue lysates; HeLa, MCF7, HEK293T, MEF, PC-12, C6 and NIH/3T3 whole cell lysates. IHC-P: Human kidney tissue; mouse and rat colon tissue. ICC/IF: HeLa

and NIH/3T3 cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate.

特記事項 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**.

#### 製品の特性

製品の状態

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR22847-50

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab238126の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000. Detects a band of approximately 94 kDa (predicted molecular weight: 94 kDa).
IHC-P		1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/30.
Flow Cyt (Intra)		1/600.

#### ターゲット情報

機能 Molecular chaperone that functions in the processing and transport of secreted proteins.

Functions in endoplasmic reticulum associated degradation (ERAD). Has ATPase activity.

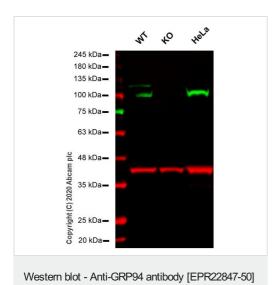
**配列類似性** Belongs to the heat shock protein 90 family.

**細胞内局在** Endoplasmic reticulum lumen. Melanosome. Identified by mass spectrometry in melanosome

fractions from stage I to stage IV.

#### 画像

(ab238126)



**All lanes :** Anti-GRP94 antibody [EPR22847-50] (ab238126) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: HSP90B1 knockout HEK293T cell lysate

Lane 3: HeLa cell lysate

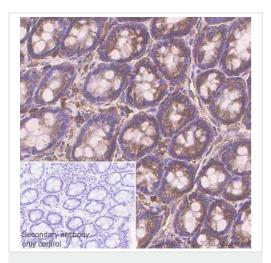
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 94 kDa **Observed band size:** 94 kDa

**Lanes 1-3:** Merged signal (red and green). Green - ab238126 observed at 94 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab238126 Anti-GRP94 antibody [EPR22847-50] was shown to specifically react with GRP94 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line <a href="mab266313"><u>ab266313</u></a> (knockout cell lysate <a href="mab257254"><u>ab257254</u></a>) was used. Wild-type and GRP94 knockout samples were subjected to SDS-PAGE. ab238126 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mab2645"><u>ab8245</u></a>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mab216773"><u>ab216773</u></a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mab216776"><u>ab216776</u></a>) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GRP94 antibody
[EPR22847-50] (ab238126)

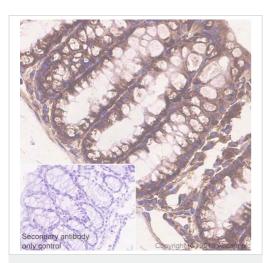
Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling GRP94 with ab238126 at 1/5000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (<a href="mailto:ab209101">ab209101</a>). Cytoplasmic staining on rat colon (PMID: 23572575) is observed. Counterstained with hematoxylin.

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

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238126 for 15 mins at RT.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GRP94 antibody
[EPR22847-50] (ab238126)

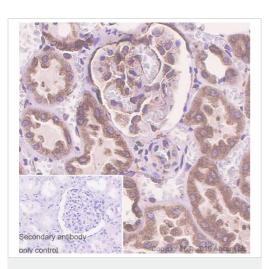
Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling GRP94 with ab238126 at 1/5000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (<a href="mailto:ab209101">ab209101</a>). Cytoplasmic staining on mouse colon (PMID: 23572575) is observed. Counterstained with hematoxylin.

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

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238126 for 15 mins at RT.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GRP94 antibody
[EPR22847-50] (ab238126)

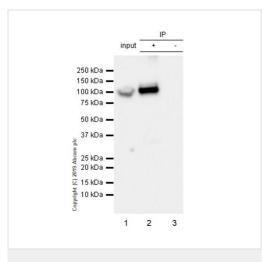
Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling GRP94 with ab238126 at 1/5000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on human kidney (PMID: 20520781) is observed. Counterstained with hematoxylin.

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

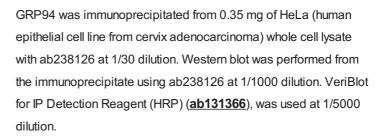
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238126 for 15 mins at RT.

The immunostaining was performed on a Leica Biosystems  $\mathsf{BOND}^{\circledR}$  RX instrument.



Immunoprecipitation - Anti-GRP94 antibody [EPR22847-50] (ab238126)

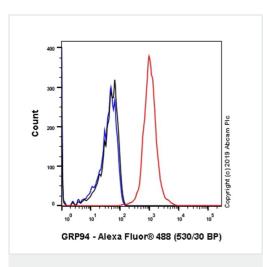


Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: ab238126 IP in HeLa whole cell lysate.

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab238126 in HeLa whole cell lysate.

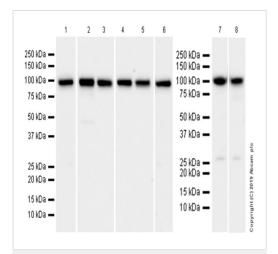
Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 seconds.



Flow Cytometry (Intracellular) - Anti-GRP94 antibody [EPR22847-50] (ab238126)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling GRP94 with ab238126 at 1/600 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>), at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-GRP94 antibody [EPR22847-50] (ab238126)

**All lanes :** Anti-GRP94 antibody [EPR22847-50] (ab238126) at 1/1000 dilution

Lane 1: Mouse brain tissue lysate at 20 µg

Lane 2 : Mouse placenta tissue lysate at 20 µg

Lane 3: Mouse stomach tissue lysate at 20 µg

Lane 4: Rat brain tissue lysate at 20 µg

Lane 5: Rat placenta tissue lysate at 20 µg

Lane 6: Rat stomach tissue lysate at 20 µg

**Lane 7 :** C6 (rat glial tumor glial cell line) whole cell lysate at 20  $\mu g$ 

Lane 8: NIH/3T3 (mouse embryo fibroblast cell line) whole cell

lysate at 10 µg

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 94 kDa Observed band size: 94 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times.

Lane 1: 26 seconds; Lanes 2-5: 10 seconds; Lane 6: 92 seconds;

Lanes 7-8: 6 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 26858453, 29949743).

**All lanes**: Anti-GRP94 antibody [EPR22847-50] (ab238126) at 1/1000 dilution

Lane 1: Human brain tissue lysate at 20 µg

Lane 2: Human kidney tissue lysate at 20 µg

Lane 3: Human stomach tissue lysate at 20 µg

Lane 4: HeLa (human epithelial cell line from cervix

adenocarcinoma) whole cell lysate at 20 µg

Lane 5: MCF7 (human breast adenocarcinoma cell line) whole cell

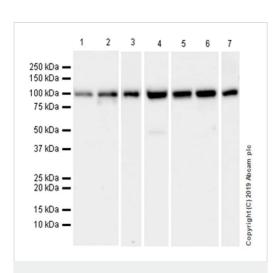
lysate at 20 µg

Lane 6 : MEF (Mouse embryonic fibroblast (immortalized) cell line)

whole cell lysate at 20 µg

Lane 7: PC-12 (rat adrenal gland pheochromocytoma cell line)

whole cell lysate at 10 µg



Western blot - Anti-GRP94 antibody [EPR22847-50] (ab238126)

#### **Secondary**

All lanes : Goat Anti-Rabbit lgG H&L (HRP) ( $\underline{ab97051}$ ) at

1/100000 dilution

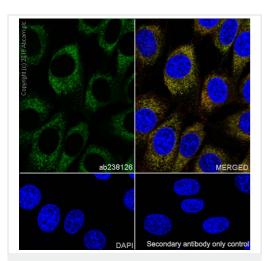
**Predicted band size:** 94 kDa **Observed band size:** 94 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times.

Lanes 1 & 2: 10 seconds; Lane 3: 37 seconds; Lanes 4-6: 10 seconds; Lane 7: 26 seconds.

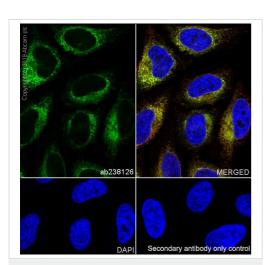
The molecular weight observed is consistent with what has been described in the literature (PMID: 26858453, 29949743).



Immunocytochemistry/ Immunofluorescence - Anti-GRP94 antibody [EPR22847-50] (ab238126)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling GRP94 with ab238126 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing endoplasmic reticulum staining in NIH/3T3 cell line. The nuclear counterstain is DAPI (blue). The endoplasmic reticulum is stained with Anti-KDEL antibody [10C3] (ab12223), followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) secondary antibody (red).

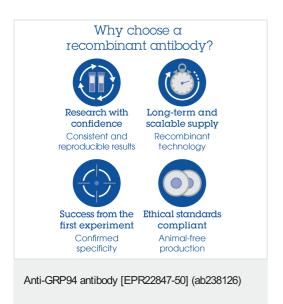
Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-GRP94 antibody [EPR22847-50] (ab238126)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling GRP94 with ab238126 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing endoplasmic reticulum staining in HeLa cell line. The nuclear counter stain is DAPI (blue). The endoplasmic reticulum is stained with Anti-KDEL antibody [10C3] (ab12223), followed by Goat Anti-Mouse lgG H&L (Alexa Fluor<sup>®</sup> 594) preadsorbed (ab150120) secondary antibody (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



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