# abcam

# Product datasheet

# Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free ab204919





重组 RabMAb

## 10 图像

#### 概述

产品名称 Anti-alpha Actinin 4抗体[EPR2533(2)] - BSA and Azide free

描述 兔单克隆抗体[EPR2533(2)] to alpha Actinin 4 - BSA and Azide free

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), IHC-P, WB, IP, ICC/IF

种属反应性 与反应: Mouse, Rat, Human

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

阳性对照 WB: Human skeletal muscle, A431, Mouse brain, Rat brain, Rat Heart, HAP1 and MFC7 Lysates

Flow Cyt(intra): HeLa IHC-P: Human breast carcinoma, Mouse and Rat liver tissues ICC/IF: HAP1

cells (HAP1-ACTN4 knockout cells used as negative cell line), MCF7 IP: HeLa cell lysate

常规说明 ab204919 is the carrier-free version of ab108198.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

#### 性能

形式

存放说明 Shipped at 4°C. Store at +4°C. Do Not Freeze.

存储溶液 pH: 7.2

Constituent: PBS

无载体 5

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR2533(2)

**同种型** IgG

#### 应用

细胞定位

The Abpromise guarantee Abpromise™承诺保证使用ab204919于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

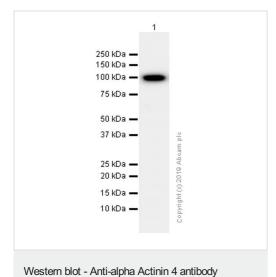
应用	Ab评论	说明
Flow Cyt (Intra)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 100 kDa (predicted molecular weight: 105 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

靶标	
功能	F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein. Probably involved in vesicular trafficking via its association with the CART complex. The CART complex is necessary for efficient transferrin receptor recycling but not for EGFR degradation.
组织 <b>特异性</b>	Widely expressed.
疾病相关	Defects in ACTN4 are the cause of focal segmental glomerulosclerosis type 1 (FSGS1) [MIM:603278]. A renal pathology defined by the presence of segmental sclerosis in glomeruli and resulting in proteinuria, reduced glomerular filtration rate and edema. Renal insufficiency often progresses to end-stage renal disease, a highly morbid state requiring either dialysis therapy or kidney transplantation.
序列相似性	Belongs to the alpha-actinin family.  Contains 1 actin-binding domain.  Contains 2 CH (calponin-homology) domains.  Contains 2 EF-hand domains.  Contains 4 spectrin repeats.

Nucleus. Cytoplasm. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Colocalizes with actin stress fibers. Nuclear translocation can be induced by the PI3 kinase inhibitor wortmannin or by cytochalasin D. Exclusively localized in the nucleus in a limited number of cell lines.

#### 图片



[EPR2533(2)] - BSA and Azide free (ab204919)

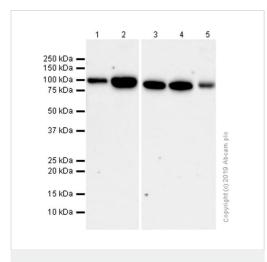
Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198) at 1/10000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15  $\mu$ g

#### **Secondary**

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

**Predicted band size:** 105 kDa **Observed band size:** 105 kDa

This data was developed using <u>ab108198</u>, the same antibody clone in a different buffer formulation.



Western blot - Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919) **All lanes :** Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198) at 1/1000 dilution (Purified)

Lane 1: Human skeletal muscle lysate

Lane 2: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate

Lane 3: Mouse brain lysate

Lane 4: Rat brain lysate

Lane 5: Rat heart lysate

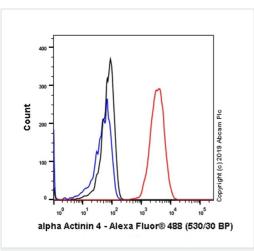
Lysates/proteins at 20 µg per lane.

#### Secondary

 $\begin{tabular}{ll} \textbf{All lanes:} Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution \end{tabular}$ 

**Predicted band size:** 105 kDa **Observed band size:** 105 kDa

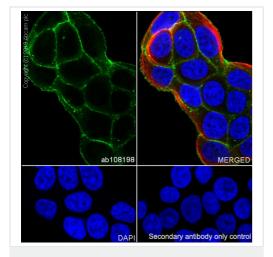
This data was developed using <u>ab108198</u>, the same antibody clone in a different buffer formulation.



Flow Cytometry (Intracellular) - Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919)

This data was developed using <u>ab108198</u>, the same antibody clone in a different buffer formulation.

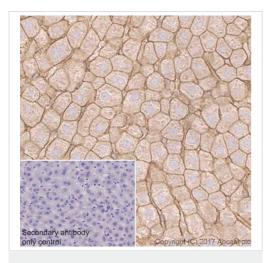
Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling alpha Actinin 4 with Purified **ab108198** at 1:50 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Antialpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919)

This data was developed using <u>ab108198</u>, the same antibody clone in a different buffer formulation.

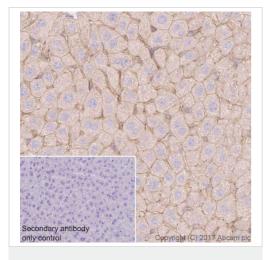
Immunocytochemistry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling alpha Actinin 4 with Purified  $\underline{ab108198}$  at 1:250 dilution (2.0 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit lgG (Alexa Fluor® 488,  $\underline{ab150077}$ ) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919)

This data was developed using **ab108198**, the same antibody clone in a different buffer formulation.

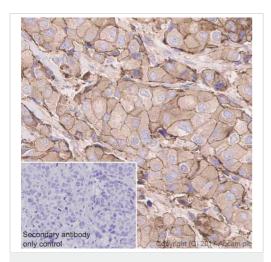
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue sections labeling alpha Actinin 4 with Purified <a href="mailto:ab108198">ab108198</a> at 1:150 dilution (3.29 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919)

This data was developed using <u>ab108198</u>, the same antibody clone in a different buffer formulation.

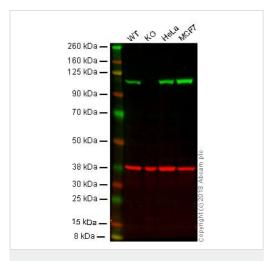
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling alpha Actinin 4 with Purified <a href="mailto:ab108198">ab108198</a> at 1:150 dilution (3.29 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Actinin 4 antibody
[EPR2533(2)] - BSA and Azide free (ab204919)

This data was developed using <u>ab108198</u>, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling alpha Actinin 4 with Purified <a href="mailto:ab108198">ab108198</a> at 1:150 dilution (3.29 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108198**).

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

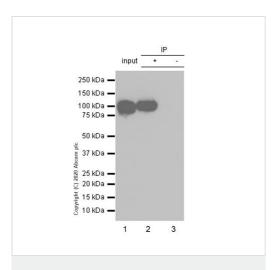
Lane 2: ACTN4 (alpha Actinin 4) knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: MCF7 whole cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab108198</u> observed at 105 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

ab108198 was shown to specifically react with alpha Actinin 4 in wild-type HAP1 cells as signal was lost in ACTN4 (alpha Actinin 4) knockout cells. Wild-type and ACTN4 (alpha Actinin 4) knockout samples were subjected to SDS-PAGE. ab108198 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-alpha Actinin 4 antibody [EPR2533(2)] - BSA and Azide free (ab204919)

Purified <u>ab108198</u> at 1/50 dilution ( $2\mu g$ ) immunoprecipitating alpha Actinin 4 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab108198 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab108198</u> in HeLa whole cell lysate.

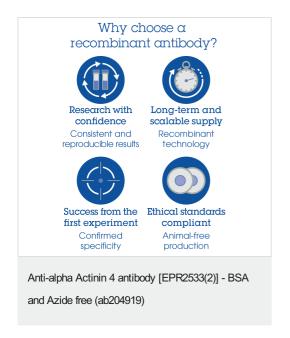
VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 105 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108198).



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