# abcam

# Product datasheet

# Anti-Cortactin antibody [EP1922Y] ab81208



ילעבע RabMAb

★★★★★ 2 Abreviews 32 References 画像数9

#### 製品の概要

製品名 Anti-Cortactin antibody [EP1922Y]

製品の詳細 Rabbit monoclonal [EP1922Y] to Cortactin

由来種 Rabbit

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

種交差性 交差種: Mouse, Human

交差が予測される動物種: Rat 4

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: HeLa and HEK-293T cell lysate. IHC-P: Human breast carcinoma. ICC/IF: MCF7 and wild-

type HAP1 cells. IP: HeLa 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

クローン名

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

バッファー pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

EP1922Y

精製度 Protein A purified

ポリモノ モノクローナル

#### アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab81208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   |
|------------------|------------------|--|
| Flow Cyt (Intra) |                  | 1/10. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.   |
| WB               |                  | 1/50000 - 1/100000. Predicted molecular weight: 62 kDa.  |
| IHC-P            |                  | 1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Heat mediated antigen retrieval with citrate buffer, pH 6 or Tris-EDTA, pH 9 are both suitable methods IHC-P with this antibody. |
| ICC/IF           | <b>★★★★★ (2)</b> | 1/1000.  |
| IP               |                  | 1/50.  |

# ターゲット情報

| 機能 Contributes to the organization of the actin cytoskeleton and cell structure. Plays a role in the |
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|--|

metastases.

**配列類似性** Contains 7 cortactin repeats.

Contains 1 SH3 domain.

ドメイン The SH3 motif may mediate binding to the cytoskeleton.

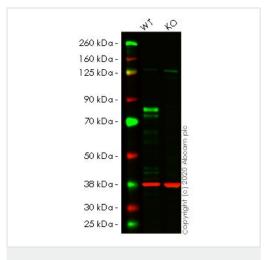
翻訳後修飾 Tyrosine phosphorylation in transformed cells may contribute to cellular growth regulation and

transformation.

細胞内局在 Cytoplasm > cytoskeleton. Cell projection > lamellipodium. Cell projection > ruffle. Associated

with membrane ruffles and lamellipodia.

#### 画像



Western blot - Anti-Cortactin antibody [EP1922Y] (ab81208)

**All lanes :** Anti-Cortactin antibody [EP1922Y] (ab81208) at 1/50000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: CTNN knockout HEK-293T cell lysate

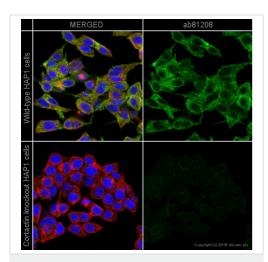
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

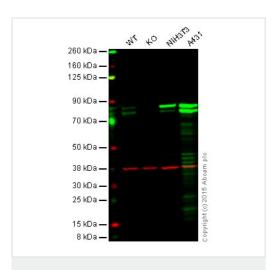
**Predicted band size:** 62 kDa **Observed band size:** 70 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab81208 observed at 70 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab81208 Anti-Cortactin antibody [EP1922Y] was shown to specifically react with Cortactin in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab266819">ab266819</a> (knockout cell lysate <a href="mailto:ab257147">ab257147</a>) was used. Wild-type and Cortactin knockout samples were subjected to SDS-PAGE. ab81208 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab8245</a>) were incubated overnight at 4°C at 1 in 50000 Dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Cortactin antibody [EP1922Y] (ab81208)



Western blot - Anti-Cortactin antibody [EP1922Y] (ab81208)

ab81208 staining Cortactin in wild-type HAP1 cells (top panel) and Cortactin knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab81208 at 1/1000 dilution and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

**All lanes :** Anti-Cortactin antibody [EP1922Y] (ab81208) at 1/2000 dilution

Lane 1: Wild-type HAP1 cell lysate

Lane 2: Cortactin knockout HAP1 cell lysate

Lane 3: NIH3T3 cell lysate
Lane 4: A431 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 62 kDa

**Lanes 1 - 4**: Merged signal (red and green). Green - ab81208 observed at 62 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab81208 was shown to specifically react with Cortactin in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when Cortactin knockout samples were examined. Wild-type and Cortactin knockout samples were subjected to SDS-PAGE. ab81208 and <a href="mailto:ab8245">ab8245</a> (loading control to GAPDH) were both diluted 1/2000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW)

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preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

1P
input + 
250 kDa —
150 kDa —
75 kDa —
50 kDa —
2d 37 kDa —
2d 37 kDa —
2d 25 kDa —
2d 25 kDa —
2d 25 kDa —
2d 25 kDa —
2d 15 kDa —
2d 15 kDa —
2d 15 kDa —
2d 20 kDa —
2d

Immunoprecipitation - Anti-Cortactin antibody [EP1922Y] (ab81208)

Purified ab81208 at 1/50 dilution (2µg) immunoprecipitating Cortactin in HeLa whole cell lysate.

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

Lane 2 (+): ab81208 + HeLa whole cell lysate.

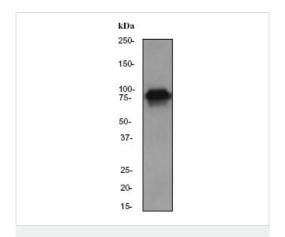
Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab81208 in HeLa whole cell lysate.

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: 62 kDa



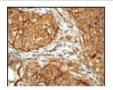
Western blot - Anti-Cortactin antibody [EP1922Y] (ab81208)

Anti-Cortactin antibody [EP1922Y] (ab81208) at 1/100000 dilution + HeLa cell lysate at 10 µg

### **Secondary**

HRP labelled Goat anti-Rabbit at 1/2000 dilution

Predicted band size: 62 kDa

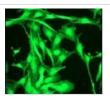


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cortactin antibody
[EP1922Y] (ab81208)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma staining Cortacin with ab81208 at 1/100 dilution. Heat mediated antigen retrieval with Tris-EDTA (pH 9) was perfomed.

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.

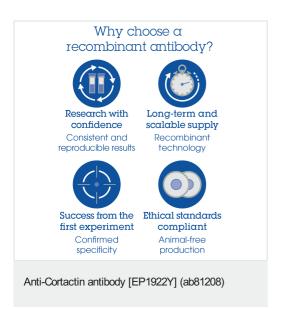
Immunofluorescent staining of MCF7 cells using 1/100 ab81208



Immunocytochemistry/ Immunofluorescence - Anti-Cortactin antibody [EP1922Y] (ab81208)

Flow Cytometry (Intracellular) - Anti-Cortactin antibody [EP1922Y] (ab81208)

Overlay histogram showing HeLa cells stained with ab81208 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab81208, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1 $\mu$ g/1x106 cells) used under the same conditions. Acquisition of >5,000 events was performed.



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