# abcam

# Product datasheet

# Anti-RYBP antibody [EPR13059(2)] - ChIP Grade ab185971

יעלאעבע RabMAb

★★★★★ 1 Abreviews 6 References 画像数 14

#### 製品の概要

製品名 Anti-RYBP antibody [EPR13059(2)] - ChIP Grade

製品の詳細 Rabbit monoclonal [EPR13059(2)] to RYBP - ChIP Grade

由来種 Rabbit

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

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 K562, SW480, HepG2 and rat PC12 cell lysates; Human placenta and Human clear carcinoma of

kidney tissues; HepG2, SW480 and K562 cells IP: NIH/3T3 cell lysates Flow Cyt (intra): A431

cells

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 Liquid

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR13059(2)

# アプリケーション

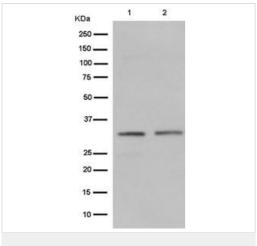
**The Abpromise guarantee Abpromise保証は、**次のテスト済みアプリケーションにおけるab185971の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/20 - 1/40. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ChIP		Use at an assay dependent concentration.
IP		1/40 - 1/60.
ICC/IF	<b>★★★★</b> <u>(1)</u>	1/250.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/1000 - 1/10000. Detects a band of approximately 32 kDa (predicted molecular weight: 25 kDa).

# ターゲット情報

機能	May be implicated in the regulation of the transcription as a repressor of the transcriptional activity of E4TF1. In tumor cell lines, may induce apoptosis.	
組織特異性	Widely expressed with highest levels in lymphoid tissues and placenta.	
配列類似性	Contains 1 RanBP2-type zinc finger.	
細胞内局在	Nucleus. Cytoplasm. Primarily found in the nucleus.	

## 画像



Western blot - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

**All lanes**: Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971) at 1/1000 dilution

Lane 1 : K562 cell lysate

Lane 2 : SW480 cell lysate

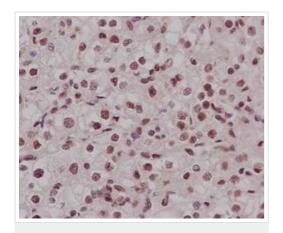
Lysates/proteins at 20 µg per lane.

### **Secondary**

**All lanes :** goat anti-rabbit lgG, (H+L), peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 25 kDa Observed band size: 32 kDa

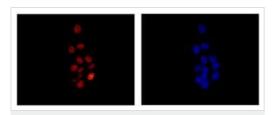


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RYBP antibody

[EPR13059(2)] - ChIP Grade (ab185971)

Immunohistochemical analysis of paraffin-embedded, Human clear cell carcinoma kidney tissue labeling RYBP with ab185971 at a 1/100 dilution. Counter stained with hematoxylin.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

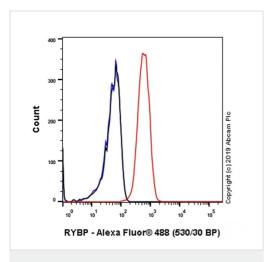


Immunocytochemistry/ Immunofluorescence - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

Immunofluorescence analysis of, paraformaldehyde-fixed, HepG2 cells labeling RYBP with ab185971 at a 1/250 dilution. As secondary antibody goat anti-rabbit lgG (Alexa Fluor®555) was used at a 1/200. In blue DAPI staining.



Immunoprecipitation - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)



Flow Cytometry (Intracellular) - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

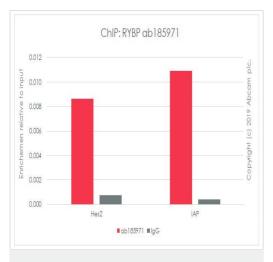
ab185971 (purified) at 1/20 dilution (11.7 $\mu$ g/mL) immunoprecipitating RYBP in NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10  $\mu$ g.

Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10  $\mu$ g Lane 2 (+): ab185971 & NIH/3T3 whole cell lysate Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab185971 in NIH/3T3 whole cell lysate

For western blotting, ab185971 at 1/500 dilution (0.468  $\mu$ g/mL) and veriBlot for IP secondary antibody (HRP) (ab131366) at 1/1000 dilution was used.

Blocking and diluting buffer: 5% NFDM /TBST.

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling RYBP with purified ab185971 at 1/20 dilution (11.7µg/mL) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) (black). Unlabeled control - Unlabelled cells (blue).



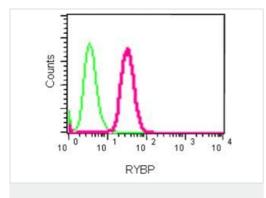
ChIP - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

Chromatin was prepared from NIH/3T3 cells according to the Abcam Dual X-ChIP protocol\*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25  $\mu$ g of chromatin, 5  $\mu$ g of ab185971 (red), and 20  $\mu$ l of Protein A/G sepharose beads. 5  $\mu$ g of rabbit normal lgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

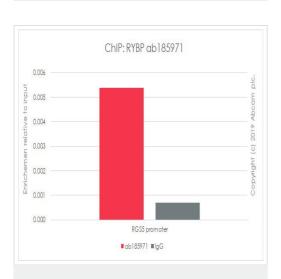
Primers and probes are located in the first kb of the transcribed region.

\*http://www.abcam.com/resources? keywords=X%20ChIP%20protocol



Flow Cytometry (Intracellular) - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

Intracellular Flow Cytometry analysis of paraformaldehyde-fixed K562 cells labeling RYBP with ab185971 at a 1/40 dilution and secondary antibody goat anti-rabbit lgG (FITC, red) at a 1/150 dilution, or negative control rabbit lgG (green).



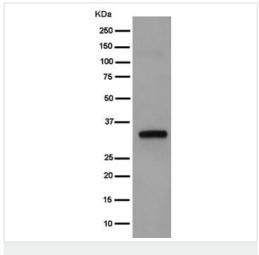
ChIP - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

Chromatin was prepared from HEK293 cells according to the Abcam Dual X-ChIP protocol\*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25  $\mu$ g of chromatin, 5  $\mu$ g of ab185971 (red), and 20  $\mu$ l of Protein A/G sepharose beads. 5  $\mu$ g of rabbit normal lgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

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Western blot - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

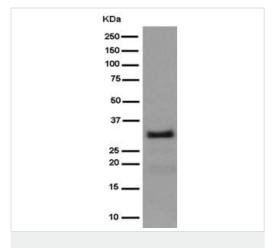
Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971) at 1/10000 dilution + HepG2 cell lysate at 20  $\mu g$ 

#### Secondary

goat anti-rabbit lgG, (H+L), peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 25 kDa Observed band size: 32 kDa



Western blot - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

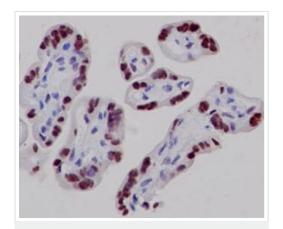
Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971) at 1/1000 dilution + rat PC12 cell lysate at 10  $\mu g$ 

### **Secondary**

goat anti-rabbit lgG, (H+L), peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

**Predicted band size:** 25 kDa **Observed band size:** 32 kDa

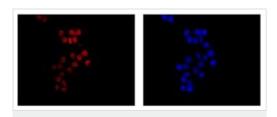


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RYBP antibody

[EPR13059(2)] - ChIP Grade (ab185971)

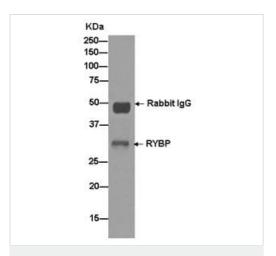
Immunohistochemical analysis of paraffin-embedded, Human placenta tissue labeling RYBP with ab185971 at a 1/100 dilution. Counter stained with hematoxylin.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



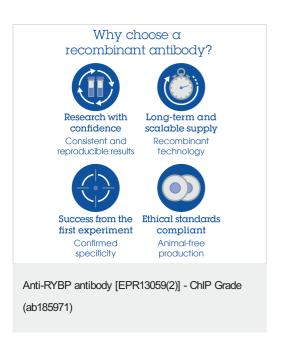
Immunocytochemistry/ Immunofluorescence - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

Immunofluorescence analysis of, paraformaldehyde-fixed, SW480 cells labeling RYBP with ab185971 at a 1/250 dilution. As secondary antibody goat anti-rabbit lgG (Alexa Fluor®555) was used at a 1/200. In blue DAPI staining.



Immunoprecipitation - Anti-RYBP antibody [EPR13059(2)] - ChIP Grade (ab185971)

Western blot analysis on immunoprecipitation pellet from HepG2 cell lysate labeling RYBP with ab185971 at 1/50 dilution and goat anti-rabbit lgG, (H+L), peroxidase conjugated at a 1/1000 dilution.



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