

# **Product datasheet**

# Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] ab289975

Recombinant RabMAb

Overview

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Overview		
Product name	Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18]	
Description	Rabbit monoclonal [EPR25290-18] to HCF-1 / Host Cell Factor C1	
Host species	Rabbit	
Tested applications	Suitable for: ICC/IF, IHC-P, WB, Flow Cyt (Intra)	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: HeLa, K562, C2C12 and C6 whole cell lysates; Mouse lung and testis tissue lysates. IHC-P: Human liver tissue; Rat liver tissue; Mouse lung tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow cyt: HeLa and NIH/3T3 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 <u>see here</u> . Our RabMAb <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u> .	

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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR25290-18
lsotype	lgG

## Applications

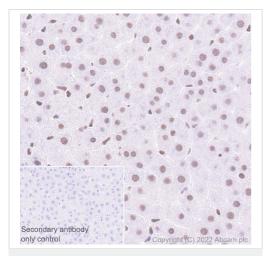
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab289975 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/2000.
IHC-P		1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 208 kDa.
Flow Cyt (Intra)		1/500.

## Target

Function	Involved in control of the cell cycle. Also antagonizes transactivation by ZBTB17 and GABP2; represses ZBTB17 activation of the p15(INK4b) promoter and inhibits its ability to recruit p300. Coactivator for EGR2 and GABP2. Tethers the chromatin modifying Set1/Ash2 histone H3 'Lys-4' methyltransferase (H3K4me) and Sin3 histone deacetylase (HDAC) complexes (involved in the activation and repression of transcription, respectively) together. In case of human herpes simplex virus (HSV) infection, HCFC1 forms a multiprotein-DNA complex with the viral transactivator protein VP16 and POU2F1 thereby enabling the transcription of the viral immediate early genes.
Tissue specificity	Highly expressed in fetal tissues and the adult kidney. Present in all tissues tested.
Sequence similarities	Contains 5 Kelch repeats.
Domain	The HCF repeat is a highly specific proteolytic cleavage signal. The kelch repeats fold into a 6-bladed kelch beta-propeller called the beta-propeller domain which mediates interaction with HCFC1R1.
Post-translational modifications	Proteolytically cleaved at one or several PPCETHET sites within the HCF repeats. Further cleavage of the primary N- and C-terminal chains results in a 'trimming' and accumulation of the smaller chains. O-glycosylated. Ubiquitinated. Lys-1807 and Lys-1808 are ubiquitinated both via 'Lys-48'- and 'Lys-63'-linked polyubiquitin chains. BAP1 mediated deubiquitination of 'Lys-48'-linked polyubiquitin chains; deubiquitination by BAP1 does not seem to stabilize the protein.
Cellular localization	Cytoplasm. Nucleus. HCFC1R1 modulates its subcellular localization and overexpression of HCFC1R1 leads to accumulation of HCFC1 in the cytoplasm. Nuclear in general, but uniquely cytoplasmic in trigeminal ganglia, becoming nuclear upon HSV reactivation from the latent state.

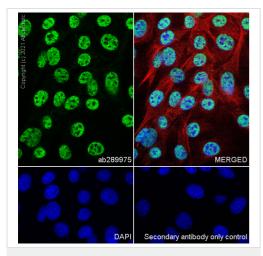


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975)

Immunohistochemical analysis of paraffin-embedded Rat liver tissue labelling HCF-1 / Host Cell Factor C1 with ab289975 at 1/10000 dilution followed by Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on rat liver. The section was incubated with ab289975 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

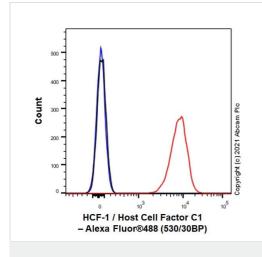
Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

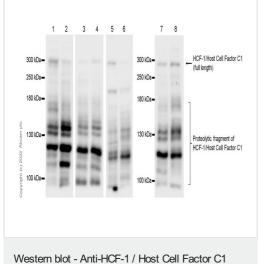


Immunocytochemistry/ Immunofluorescence - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975) Immunofluorescent analysis of 100% methanol-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 cells labeling HCF-1 / Host Cell Factor C1 with ab289975 at 1/2000 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in NIH/3T3 cell line. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution(Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975) Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling HCF-1 / Host Cell Factor C1 with ab289975 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



antibody [EPR25290-18] (ab289975)

All lanes : Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lane 2 : K562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate

Lane 3: NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

Lane 4: C2C12 (mouse myoblasts), whole cell lysate

Lane 5: C6 (rat glial tumor glial cell), whole cell lysate

Lane 6 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

- Lane 7 : Mouse lung tissue lysate
- Lane 8 : Mouse testis tissue lysate

Lysates/proteins at 20 µg per lane.

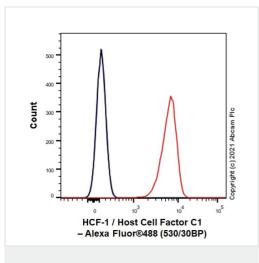
#### Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/20000 dilution

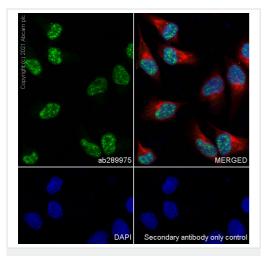
Predicted band size: 208 kDa Observed band size: 100-150,300 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 31693902)



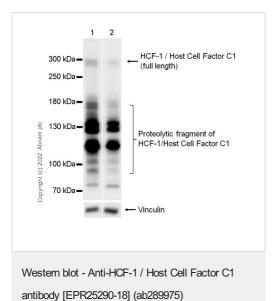
Flow Cytometry (Intracellular) - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975) Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling HCF-1 / Host Cell Factor C1 with ab289975 at 1/500 dilution ( $0.1\mu$ g) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling HCF-1 / Host Cell Factor C1 with ab289975 at 1/2000 dilution, followed by <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HeLa cell line is observed. <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubµle Marker (Alexa Flu<sup>or®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution.



All lanes : Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975) at 1/5000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) transfected with scrambled siRNA control, whole cell lysate Lane 2 : HeLa transfected with HCF-1/Host Cell Factor C1 siRNA, whole cell lysate

Lysates/proteins at 10 µg per lane.

# Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 208 kDa Observed band size: 100-150,300 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID:Â 31693902).

Exposure time: 26 seconds.

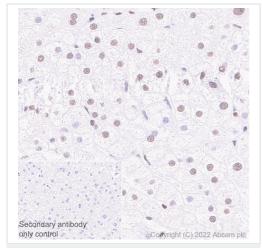


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975)

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labelling HCF-1 / Host Cell Factor C1 with ab289975 at 1/10000 followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on mouse lung. The section was incubated with ab289975 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: 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 solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HCF-1 / Host Cell Factor C1 antibody [EPR25290-18] (ab289975)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labelling HCF-1 / Host Cell Factor C1 with ab289975 at 1/5000 followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human liver. The section was incubated with ab289975 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: 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 solution2) for 20 mins



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