# abcam

# Product datasheet

# Anti-IRF5 antibody [EPR17067] ab181553



★★★★★★ 2 Abreviews 17 References

画像数 18

#### 製品の概要

製品名 Anti-IRF5 antibody [EPR17067]

製品の詳細 Rabbit monoclonal [EPR17067] to IRF5

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, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: IRF5 transfected HEK293T whole cell lysate; Ramos, THP-1, A20 and RAW 264.7 whole cell

> lysates; Mouse thymus, rat thymus, mouse spleen and rat spleen lysates. IHC-P: Human spleen, Human breast cancer, Human lung cancer, Human tonsil, mouse liver and rat spleen tissues. ICC/IF: RAW 264.7 and THP-1 cells. Flow Cyt (intra): THP-1 cells. IP: RAW 264.7 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 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 Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 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 EPR17067

#### 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ab181553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)		1/50. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/1000. Detects a band of approximately 55 kDa (predicted molecular weight: 55 kDa).
IHC-P	****(1)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/150.

#### ターゲット情報

#### 関連疾患

Genetic variations in IRF5 are associated with susceptibility to inflammatory bowel disease type 14 (IBD14) [MIM:612245]. IBD14 is a chronic, relapsing inflammation of the gastrointestinal tract with a complex etiology. It is subdivided into Crohn disease and ulcerative colitis phenotypes. Crohn disease may affect any part of the gastrointestinal tract from the mouth to the anus, but most frequently it involves the terminal ileum and colon. Bowel inflammation is transmural and discontinuous; it may contain granulomas or be associated with intestinal or perianal fistulas. In contrast, in ulcerative colitis, the inflammation is continuous and limited to rectal and colonic mucosal layers; fistulas and granulomas are not observed. Both diseases include extraintestinal inflammation of the skin, eyes, or joints.

Genetic variations in IRF5 are associated with susceptibility to systemic lupus erythematosus type 10 (SLEB10) [MIM:612251]. Systemic lupus erythematosus (SLE) is a chronic, inflammatory and often febrile multisystemic disorder of connective tissue. It affects principally the skin, joints, kidneys and serosal membranes. It is thought to represent a failure of the regulatory mechanisms of the autoimmune system.

Genetic variations in IRF5 are a cause of susceptibility to rheumatoid arthritis (RA) [MIM:180300]. It is a systemic inflammatory disease with autoimmune features and a complex genetic component. It primarily affects the joints and is characterized by inflammatory changes in the synovial membranes and articular structures, widespread fibrinoid degeneration of the collagen fibers in mesenchymal tissues, and by atrophy and rarefaction of bony structures.

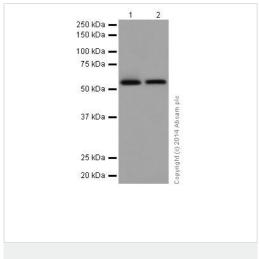
#### 配列類似性

Belongs to the IRF family.

Contains 1 IRF tryptophan pentad repeat DNA-binding domain.

#### 細胞内局在

Nucleus.



Western blot - Anti-IRF5 antibody [EPR17067] (ab181553)



Lane 1 : Mouse thymus lysate

Lane 2: Rat thymus lysate

Lysates/proteins at 10 µg per lane.

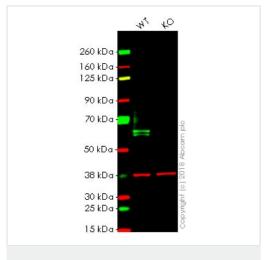
# **Secondary**

**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 55 kDa **Observed band size:** 55 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-IRF5 antibody [EPR17067] (ab181553)

**All lanes :** Anti-IRF5 antibody [EPR17067] (ab181553) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: IRF5 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

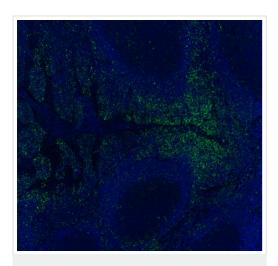
Predicted band size: 55 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - ab181553 observed at 56 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab181553 was shown to specifically react with IRF5 in wild-type HAP1 cells as signal was lost in IRF5 knockout cells. Wild-type and IRF5 knockout samples were subjected to SDS-PAGE. Ab181553 and <a href="mailto:ab9484">ab9484</a> (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

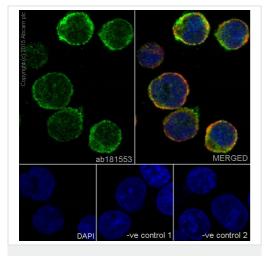
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(IRDye $^{\$}$  800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye $^{\$}$  680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRF5 antibody
[EPR17067] (ab181553)

Anti-IRF5 antibody [EPR17067] (ab181553)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling IRF5 with ab181553 at a dilution of 1:500. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (PerkinElmer Opal Polymer HRP Ms Plus Rb) was used as secondary antibody. Opal tyramide amplification was performed using Opal 520 fluorophore. Counterstained with DAPI stain. Image scanned with Vectra 3.0 and analyzed via Phenochart software. This image was courteously provided by Dr. Houssein Abdul Sater, Georgia Cancer Center.

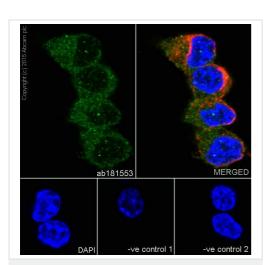


Immunocytochemistry/ Immunofluorescence - Anti-IRF5 antibody [EPR17067] (ab181553)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized THP-1 (Human monocytic leukemia cells) cells labeling IRF5 with ab181553 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and weakly nuclear staining on THP-1 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab181553 at 1/1000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.

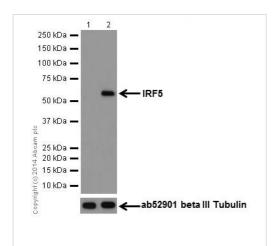


Immunocytochemistry/ Immunofluorescence - Anti-IRF5 antibody [EPR17067] (ab181553)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) cells labeling IRF5 with ab181553 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and weakly nuclear staining on RAW 264.7 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab181553 at 1/1000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



Western blot - Anti-IRF5 antibody [EPR17067] (ab181553)

**All lanes :** Anti-IRF5 antibody [EPR17067] (ab181553) at 1/5000 dilution

**Lane 1 :** Non-transfected HEK293T (Human epithelial cells from embryonic kidney) whole cell lysate

**Lane 2**: IRF5 transfected HEK293T (Human epithelial cells from embryonic kidney) whole cell lysate

Lysates/proteins at 10 µg per lane.

### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 55 kDa **Observed band size:** 55 kDa

Exposure time: 1 minute

Transfected lysate from abcam (Cat#:ab94265).

Blocking/Dilution buffer: 5% NFDM/TBST.

250 kDa — 150 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa —

Western blot - Anti-IRF5 antibody [EPR17067] (ab181553)

Anti-IRF5 antibody [EPR17067] (ab181553) at 1/2000 dilution + Ramos (Human Burkitt's lymphoma cell line) whole cell lysate at 10 µg

# Secondary

Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 55 kDa Observed band size: 55 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 250 kDa — 150 kDa — 100 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa —

Western blot - Anti-IRF5 antibody [EPR17067] (ab181553)

**All lanes :** Anti-IRF5 antibody [EPR17067] (ab181553) at 1/50000 dilution

Lane 1 : THP-1 (Human monocytic leukemia cells) whole cell lysateLane 2 : A20 (Mouse reticulum cell sarcoma cell line) whole celllysate

Lysates/proteins at 10 µg per lane.

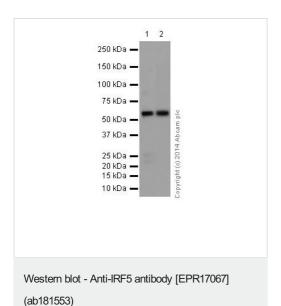
#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 55 kDa **Observed band size:** 55 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



**All lanes :** Anti-IRF5 antibody [EPR17067] (ab181553) at 1/2000 dilution

Lane 1: Mouse spleen lysate

Lane 2: Rat spleen lysate

Lysates/proteins at 10 µg per lane.

# Secondary

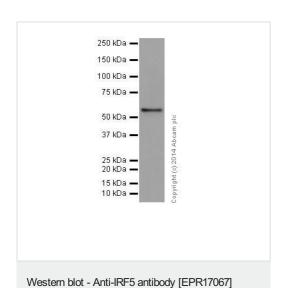
 $\textbf{All lanes:} \ \ \text{Goat Anti-Rabbit lgG, (H+L),} Peroxidase \ conjugated \ at$ 

1/1000 dilution

Predicted band size: 55 kDa Observed band size: 55 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



RAW 264.7(Mouse macrophage cells transformed with Abelson murine leukemia virus ) whole cell lysate at 10  $\mu g$ 

#### Secondary

Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

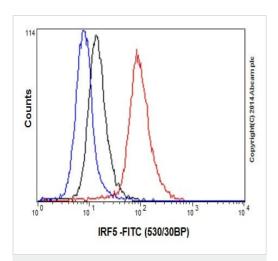
Anti-IRF5 antibody [EPR17067] (ab181553) at 1/5000 dilution +

**Predicted band size:** 55 kDa **Observed band size:** 55 kDa

Exposure time: 5 seconds

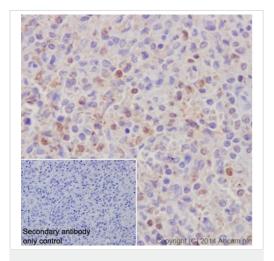
(ab181553)

Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-IRF5 antibody [EPR17067] (ab181553)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed THP-1 (Human monocytic leukemia cells) cells labeling IRF5with ab181553 at 1/50 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730;black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



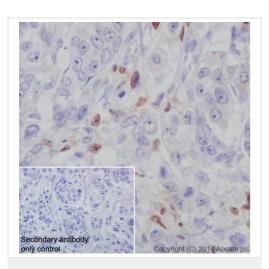
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRF5 antibody
[EPR17067] (ab181553)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling IRF5 with ab181553 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and cytoplasmic staining on leukocytes of Human spleen is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP)

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

(ab97051) at 1/500 dilution.

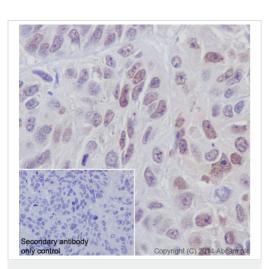


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRF5 antibody
[EPR17067] (ab181553)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling IRF5 with ab181553 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and cytoplasmic staining on leukocyte of Human breast cancer stroma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

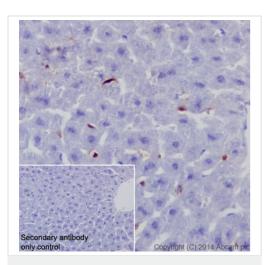


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRF5 antibody
[EPR17067] (ab181553)

Immunohistochemical analysis of paraffin-embedded Human lung cancer tissue labeling IRF5 with ab181553 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nucleus staining on cancer cells of Human lung cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

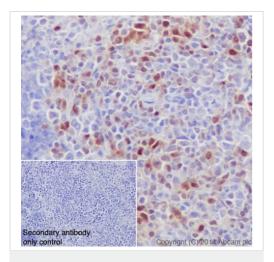


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRF5 antibody
[EPR17067] (ab181553)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling IRF5 with ab181553 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Nuclear staining on kupffer cells of mouse liver is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

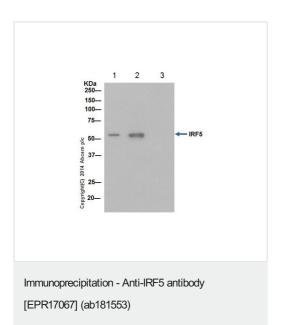


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRF5 antibody
[EPR17067] (ab181553)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling IRF5 with ab181553 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and cytoplasmic staining on lymphocytes of rat spleen is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



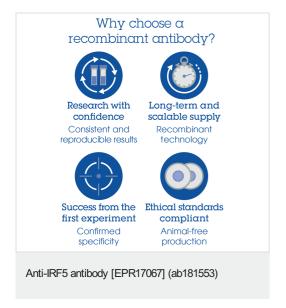
IRF5 was immunoprecipitated from 1mg of RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate with ab181553 at 1/150 dilution. Western blot was performed from the immunoprecipitate using ab181553 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: RAW 264.7 whole cell lysate 10 µg (Input).

Lane 2: ab181553 IP in RAW 264.7 whole cell lysate.

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of ab181553 in RAW 264.7 whole cell lysate.

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



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