abcam

Product datasheet

Anti-BTF3 antibody [EPR16495] ab203517

Recombinant RabMAb

★★★★★ 1 Abreviews 3 References 15 Images

Overview

Product name Anti-BTF3 antibody [EPR16495]

Description Rabbit monoclonal [EPR16495] to BTF3

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: MCF7, SK-BR-3, HeLa, Jurkat, C6, RAW 264.7, PC-12 and NIH/3T3 cell lysates; Human

> lymph node and fetal kidney lysates; Mouse heart and spleen lysates. IHC-P: Human cervix carcinoma, Human spleen, mouse liver and rat testis tissues. ICC/IF: HeLa and SK-BR-3 cells.

Flow Cyt (intra): HeLa cells. IP: NIH/3T3 whole cell lysate.

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 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**.

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: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR16495

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab203517 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
Flow Cyt (Intra)		1/250.
WB	**** <u>(1)</u>	1/1000. Detects a band of approximately 22, 18 kDa (predicted molecular weight: 22 kDa).
ICC/IF		1/250.
IP		1/70.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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Function General transcription factor. BTF3 can form a stable complex with RNA polymerase II. Required

for the initiation of transcription.

Sequence similaritiesBelongs to the NAC-beta family.

Contains 1 NAC-A/B (NAC-alpha/beta) domain.

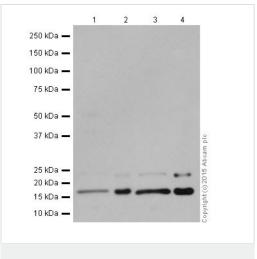
Post-translational

Phosphorylated upon DNA damage, probably by ATM or ATR.

modifications

Cellular localization Nucleus.

Images



Western blot - Anti-BTF3 antibody [EPR16495] (ab203517)

All lanes : Anti-BTF3 antibody [EPR16495] (ab203517) at 1/2000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma cell line) cell lysate

Lane 2: SK-BR-3 (Human mammary gland adenocarcinoma cell line) cell lysate

Lane 3 : HeLa (Human epithelial cells from cervix adenocarcinoma) cell lysate

Lane 4 : Jurkat (Human T cell leukemia cells from peripheral blood) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

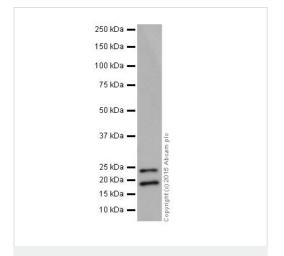
All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 22 kDa

Observed band size: 18,22 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-BTF3 antibody [EPR16495] (ab203517)

Anti-BTF3 antibody [EPR16495] (ab203517) at 1/1000 dilution + Human fetal kidney lysate at 10 μg

Secondary

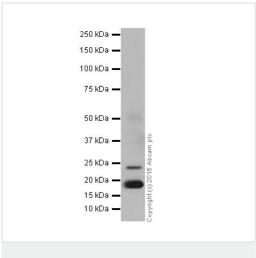
Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/10000 dilution

Predicted band size: 22 kDa

Observed band size: 18,22 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-BTF3 antibody [EPR16495] (ab203517)

Anti-BTF3 antibody [EPR16495] (ab203517) at 1/1000 dilution + C6 (Rat glial tumor cells) cell lysate at 10 μg

Secondary

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

Predicted band size: 22 kDa **Observed band size:** 18,22 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

15 kDa —

Western blot - Anti-BTF3 antibody [EPR16495] (ab203517)

Anti-BTF3 antibody [EPR16495] (ab203517) at 1/1000 dilution + Human lymph node lysate at 10 μg

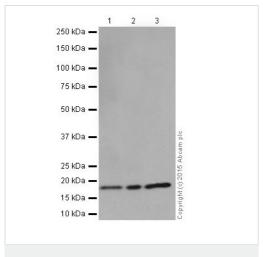
Secondary

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

Predicted band size: 22 kDa **Observed band size:** 18 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-BTF3 antibody [EPR16495] (ab203517)

All lanes : Anti-BTF3 antibody [EPR16495] (ab203517) at 1/1000 dilution

Lane 1 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) cell lysate

Lane 2: PC-12 (Rat adrenal gland pheochromocytoma) cell lysate

Lane 3: NIH/3T3 (Mouse embyro fibroblast cells) 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: 22 kDa Observed band size: 18 kDa

Exposure time: 1 minute

All lanes : Anti-BTF3 antibody [EPR16495] (ab203517) at 1/1000 dilution

Lane 1 : Mouse heart lysate

Lane 2 : Mouse spleen lysate

Lysates/proteins at 10 µg per lane.

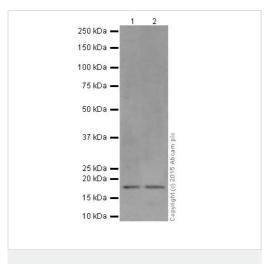
Secondary

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

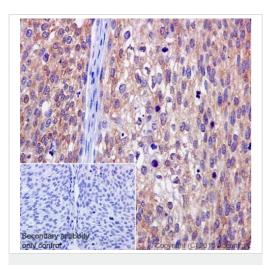
Predicted band size: 22 kDa **Observed band size:** 18 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-BTF3 antibody [EPR16495] (ab203517)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BTF3 antibody
[EPR16495] (ab203517)

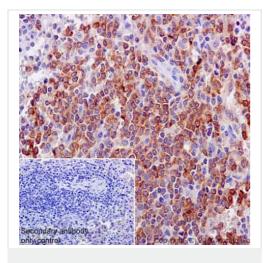
Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma labeling BTF3 with ab203517 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on Human cervix carcinoma tissue 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-BTF3 antibody
[EPR16495] (ab203517)

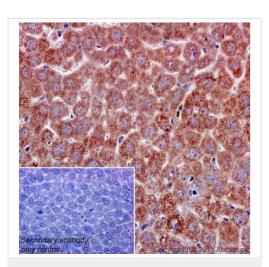
Immunohistochemical analysis of paraffin-embedded Human spleen labeling BTF3 with ab203517 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on Human spleen tissue 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-BTF3 antibody
[EPR16495] (ab203517)

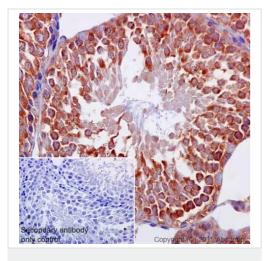
Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling BTF3 with ab203517 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on mouse liver tissue 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) (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-BTF3 antibody
[EPR16495] (ab203517)

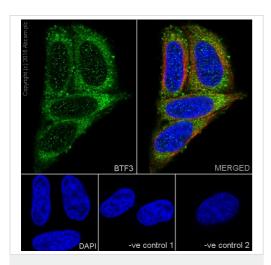
Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling BTF3 with ab203517 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on rat testis tissue 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.



Immunocytochemistry/ Immunofluorescence - Anti-BTF3 antibody [EPR16495] (ab203517)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling BTF3 with ab203517 at 1/250 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

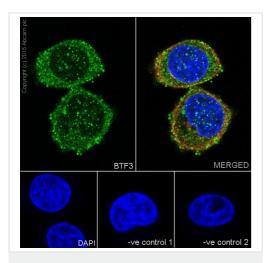
Confocal imaging showing cytoplasmic and weakly nuclear staining on HeLa cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab203517 at 1/250 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 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 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-BTF3 antibody [EPR16495] (ab203517)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-BR-3 (Human mammary gland adenocarcinoma cell line) cells labeling BTF3 with ab203517 at 1/250 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

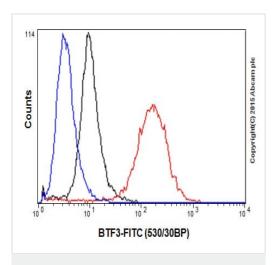
Confocal imaging showing cytoplasmic and weakly nuclear staining on SK-BR-3 cell line.

The nuclear counterstain is DAPI (blue).

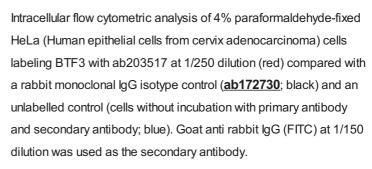
Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

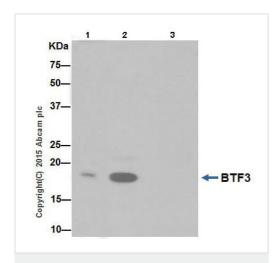
The negative controls are as follows:-

-ve control 1: ab203517 at 1/250 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 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 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-BTF3 antibody [EPR16495] (ab203517)





Immunoprecipitation - Anti-BTF3 antibody [EPR16495] (ab203517)

BTF3 was immunoprecipitated from 1mg of NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate with ab203517 at 1/70 dilution.

Western blot was performed from the immunoprecipitate using ab203517 at 1/1000 dilution.

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

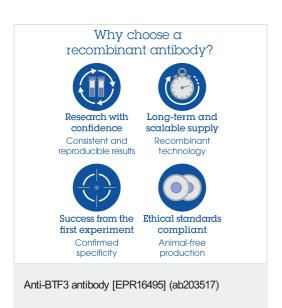
Lane 1: NIH/3T3 whole cell lysate 10ug (Input).

Lane 2: ab203517 IP in NIH/3T3 whole cell lysate.

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of ab203517 in NIH/3T3 whole cell lysate.

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

Exposure time: 10 seconds.



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