# abcam

# Product datasheet

# Anti-Frataxin antibody [EPR21840] - BSA and Azide free ab236463

Recombinant RabMAb

### 12 Images

#### Overview

**Product name** Anti-Frataxin antibody [EPR21840] - BSA and Azide free

**Description** Rabbit monoclonal [EPR21840] to Frataxin - BSA and Azide free

**Host species** Rabbit

Specificity We suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or

higher sensitivity ECL substrate) to improve results.

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HCT 116, Neuro-2a, C6, PC-12, human liver and human hippocampus tissue lysates,

> HCT116, Huh7 and SH-SY5Y whole cell lysates. IHC-P: Human testis and liver tissue; Mouse and rat kidney tissue. ICC/IF: HCT 116 and Neuro-2a cells. Flow Cyt (intra): HCT 116 cells. IP: HCT

116 and Neuro-2a cells.

General notes ab236463 is the carrier-free version of ab219414.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes,

oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP,

biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the

need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

#### **Properties**

**Form** Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

**Purity** Protein A purified

Clonality Monoclonal
Clone number EPR21840

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab236463 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)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 14 kDa (predicted molecular weight: 23 kDa).

# Target

**Function** 

Promotes the biosynthesis of heme and assembly and repair of iron-sulfur clusters by delivering Fe(2+) to proteins involved in these pathways. May play a role in the protection against iron-catalyzed oxidative stress through its ability to catalyze the oxidation of Fe(2+) to Fe(3+); the oligomeric form but not the monomeric form has in vitro ferroxidase activity. May be able to store large amounts of iron in the form of a ferrihydrite mineral by oligomerization; however, the physiological relevance is unsure as reports are conflicting and the function has only been shown using heterologous overexpression systems. Modulates the RNA-binding activity of ACO1.

Tissue specificity

Involvement in disease

Expressed in the heart, peripheral blood lymphocytes and dermal fibroblasts.

Defects in FXN are the cause of Friedreich ataxia (FRDA) [MIM:229300]. FRDA is an autosomal recessive, progressive degenerative disease characterized by neurodegeneration and cardiomyopathy it is the most common inherited ataxia. The disorder is usually manifest before adolescence and is generally characterized by incoordination of limb movements, dysarthria, nystagmus, diminished or absent tendon reflexes, Babinski sign, impairment of position and vibratory senses, scoliosis, pes cavus, and hammer toe. In most patients, FRDA is due to GAA triplet repeat expansions in the first intron of the frataxin gene. But in some cases the disease is due to mutations in the coding region.

#### Sequence similarities

Belongs to the frataxin family.

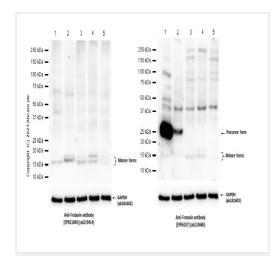
# Post-translational modifications

Processed in two steps by mitochondrial processing peptidase (MPP). MPP first cleaves the precursor to intermediate form and subsequently converts the intermediate to yield frataxin mature form (frataxin(81-210)) which is the predominant form. The additional forms, frataxin(56-210) and frataxin(78-210), seem to be produced when the normal maturation process is impaired; their physiological relevance is unsure.

#### **Cellular localization**

Cytoplasm. Mitochondrion. PubMed:18725397 reports localization exclusively in mitochondria.

#### **Images**



Western blot - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)

All lanes: ab219414 and ab124680 at 1/1000 dilution

Lane 1: Human liver tissue lysate

Lane 2: Human hippocampus tissue lysate

Lane 3: HCT116 (human colorectal carcinoma epithelial cell)

whole cell lysate

Lane 4: Huh7 (human hepatocellular carcinoma epithelial cell)

whole cell lysate

Lane 5: SH-SY5Y (human neuroblastoma epithelial cell) whole cell

lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 23 kDa

**Observed band size:** 14,17 kDa

Exposure time: 60 seconds

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

**Diluting buffer and concentration:** 5% NFDM/TBST.

This blot was developed using a high sensitivity ECL substrate.

ab219414 is not suitable for testing precursor form, we recommend

ab124680 as an alternative for precursor form testing.

For different forms of frataxin, you can refer to PMID: 17468497,

PMID: 31279523, PMID: 17468497 etc.

ab181602 was used as a loading control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab219414</u>).

Anti-Frataxin antibody [EPR21840] (ab219414) at 1/1000 dilution + His tagged Human FXN recombinant protein (aa 82-210), 10ng

### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 23 kDa **Observed band size:** 16 kDa

Exposure time: 3 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219414).

Blocking and diluting buffer and concentration: 5% NFDM/TBST. **ab213204** was used as laoding control.

Frataxin was immunoprecipitated from 0.35 mg Neuro-2a (mouse neuroblastoma cell line) whole cell lysate with **ab219414** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab219414** at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: Neuro-2a whole cell lysate 10 µg (Input).

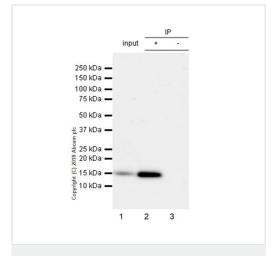
Lane 2: ab219414 IP in Neuro-2a whole cell lysate (+).

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab219414</u> in Neuro-2a whole cell lysate (-).

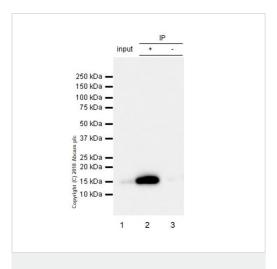
Blocking/Dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 30 seconds.



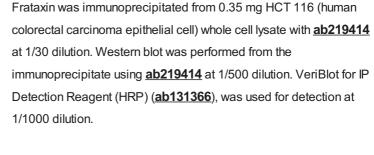
Western blot - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)



Immunoprecipitation - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)



Immunoprecipitation - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)



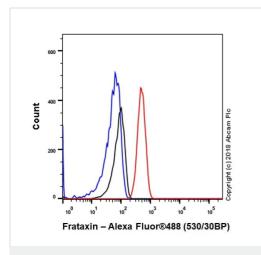
Lane 1: HCT 116 whole cell lysate 10 µg (Input).

Lane 2: ab219414 IP in HCT 116 whole cell lysate (+).

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab219414</u> in HCT 116 whole cell lysate (-).

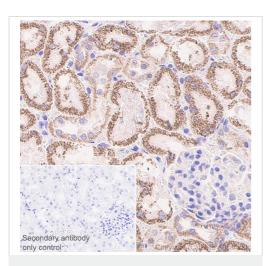
Blocking/Dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 5 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219414).



Flow Cytometry (Intracellular) - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HCT 116 (human colorectal carcinoma epithelial cell) cell line labeling Frataxin with <u>ab219414</u> at 1/60 (red) compared with a rabbit monoclonal lgG (<u>ab172730</u>) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, <u>ab150077</u>), at 1/2000 dilution was used as the secondary antibody.

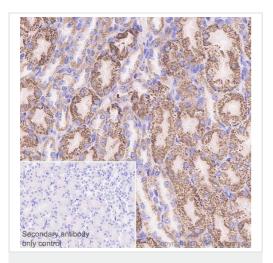


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Frataxin antibody
[EPR21840] - BSA and Azide free (ab236463)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling Frataxin with <u>ab219414</u> at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Granular cytoplasmic staining in rat kidney (PMID: 18725397; PMID: 26035392) is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat-mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer pH 9.0).

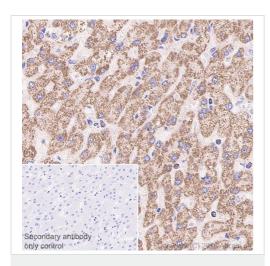
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219414).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Frataxin antibody
[EPR21840] - BSA and Azide free (ab236463)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling Frataxin with <u>ab219414</u> at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Granular cytoplasmic staining in mouse kidney (PMID: 18725397; PMID: 26035392) is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat-mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer pH 9.0).

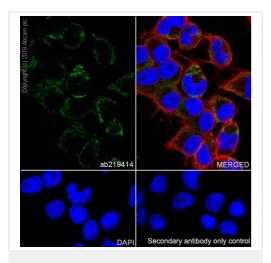


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Frataxin antibody
[EPR21840] - BSA and Azide free (ab236463)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling Frataxin with **ab219414** at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Granular cytoplasmic staining in human liver (PMID: 18725397; PMID: 26035392) is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat-mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer pH 9.0).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219414).



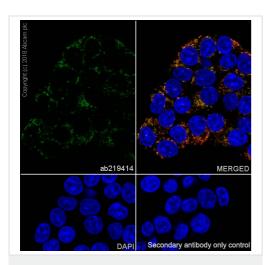
Immunocytochemistry/ Immunofluorescence - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Neuro-2a (mouse neuroblastoma cell line) cells labeling Frataxin with <u>ab219414</u> at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green).

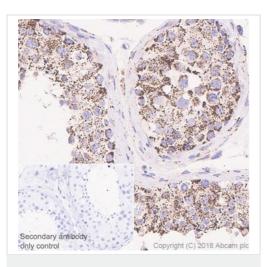
Confocal image showing cytoplasmic staining in the Neuro-2a cell line.

Counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) at a 1/200 dilution (red). The nuclear counter stain is DAPI (blue).

The negative control is the secondary antibody only.



Immunocytochemistry/ Immunofluorescence - Anti-Frataxin antibody [EPR21840] - BSA and Azide free (ab236463)



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

[EPR21840] - BSA and Azide free (ab236463)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (human colorectal carcinoma epithelial cell) cells labeling Frataxin with <u>ab219414</u> at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing mitochondrial staining in HCT 116 cell line.

Counterstained with <u>ab33985</u> Anti-COX IV antibody - Mitochondrial Marker at a 1/1000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) (<u>ab150120</u>) (orange). The nuclear counter stain is DAPI (blue).

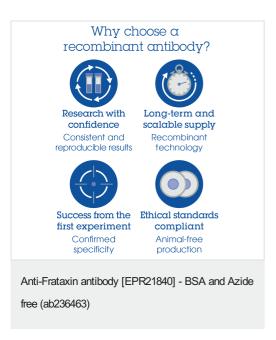
The negative control is the secondary antibody only.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219414).

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling Frataxin with <u>ab219414</u> at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Granular cytoplasmic staining in human testis (PMID: 18725397; PMID: 26035392) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Perform heat-mediated antigen retrieval using  $\underline{ab93684}$  (Tris/EDTA buffer pH 9.0).



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