# abcam

# Product datasheet

# Anti-FADD antibody [EPR5030] - BSA and Azide free ab229444



## 7 Images

#### Overview

Product name Anti-FADD antibody [EPR5030] - BSA and Azide free

**Description** Rabbit monoclonal [EPR5030] to FADD - BSA and Azide free

Host species Rabbit

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

Species reactivity Reacts with: Mouse

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Mouse kidney tissue; ICC/IF: NIH/3T3 cells; Flow Cyt (intra): NIH/3T3 cells. WB: NIH/3T3

whole cell lysate. IP: NIH/3T3 whole cell lysate and RAW264.7 whole cell lysate.

**General notes** ab229444 is the carrier-free version of **ab124812**.

Our <u>carrier-free</u> 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 cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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Human, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

#### **Properties**

Form Liquid

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

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR5030

**Isotype** IgG

### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab229444 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		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 28 kDa (predicted molecular weight: 23 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.

### **Target**

**Function** Apoptotic adaptor molecule that recruits caspase-8 or caspase-10 to the activated Fas (CD95)

or TNFR-1 receptors. The resulting aggregate called the death-inducing signaling complex (DISC)  $\,$ 

performs caspase-8 proteolytic activation. Active caspase-8 initiates the subsequent cascade of

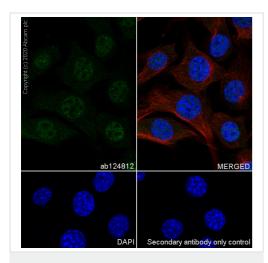
caspases mediating apoptosis.

**Tissue specificity** Expressed in a wide variety of tissues, except for peripheral blood mononuclear leukocytes.

Sequence similarities Contains 1 death domain.

Contains a death domain involved in the binding of the corresponding domain within Fas receptor.

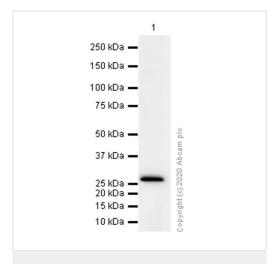
### **Images**



Immunocytochemistry/ Immunofluorescence - Anti-FADD antibody [EPR5030] - BSA and Azide free (ab229444)

This data was developed using <u>ab124812</u>, the same antibody clone in a different buffer formulation.

Immunocytochemistry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling FADD with purified <u>ab124812</u> at 1/50 dilution (7.8 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor  $^{(8)}$  594) 1/200 (2.5 µg/mL). Goat anti rabbit lgG (Alexa Fluor  $^{(8)}$  488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



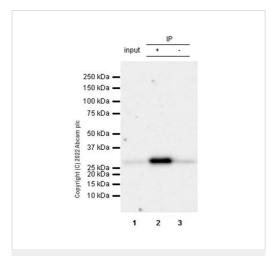
Western blot - Anti-FADD antibody [EPR5030] - BSA and Azide free (ab229444)

Anti-FADD antibody [EPR5030] (ab124812) at 1/1000 dilution (Purified) + NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

#### Secondary

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

Predicted band size: 23 kDa



Immunoprecipitation - Anti-FADD antibody
[EPR5030] - BSA and Azide free (ab229444)

FADD was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10  $\mu$ g with <u>ab124812</u> at 1/30 dilution (2  $\mu$ g in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using <u>ab124812</u> at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 µg.

Lane 2: NIH/3T3 whole cell lysate.

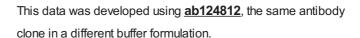
**Lane 3**: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab124812</u> in NIH/3T3 whole cell lysate.

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

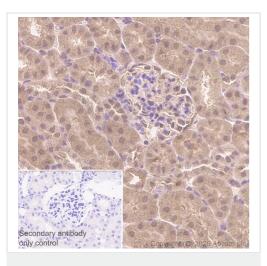
Observed MW: 28 kDa.

Exposure time: 41 secs.

This data was developed using <u>ab124812</u>, the same antibody clone in a different buffer formulation.

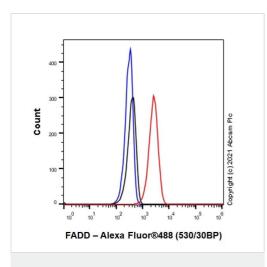


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling FADD with purified <a href="mailto:ab124812">ab124812</a> at 1/150 dilution (2.59 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.



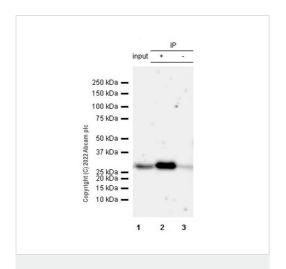
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FADD antibody

[EPR5030] - BSA and Azide free (ab229444)



Flow Cytometry (Intracellular) - Anti-FADD antibody [EPR5030] - BSA and Azide free (ab229444)

This data was developed using <u>ab124812</u>, the same antibody clone in a different buffer formulation. Intracellular Flow Cytometry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labelling FADD with purified <u>ab124812</u> at 1/40 dilution (10 "¿½g/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor"¿½488, <u>ab150077</u>) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunoprecipitation - Anti-FADD antibody [EPR5030] - BSA and Azide free (ab229444)

FADD was immunoprecipitated from 0.35 mg RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate 10 μg with <u>ab124812</u> at 1/30 dilution (2 μg in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using <u>ab124812</u> at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

**Lane 1**: RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate 10  $\mu g$ .

Lane 2: RAW264.7 whole cell lysate.

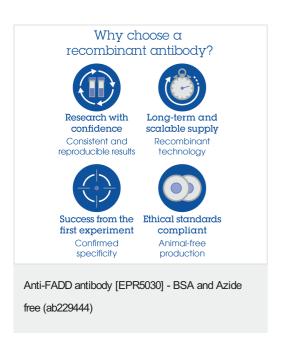
**Lane 3**: Rabbit monoclonal  $\lg G$  (<u>ab172730</u>) instead of <u>ab124812</u> in RAW264.7 whole cell lysate.

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

Observed MW: 28 kDa.

Exposure time: 3 minutes.

This data was developed using <u>ab124812</u>, the same antibody clone in a different buffer formulation.



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