

Product datasheet

Anti-DGKZ/DGK-zeta antibody [EPR22040-80] ab239081

Recombinant RabMAb

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Overview

Product name	Anti-DGKZ/DGK-zeta antibody [EPR22040-80]
Description	Rabbit monoclonal [EPR22040-80] to DGKZ/DGK-zeta
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), mIHC, IHC-P, WB, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat and HuT-78 whole cell lysates; Mouse brain, cerebellum and thymus lysates; Rat brain and cerebellum lysates. IHC-P: Human tonsil and cerebellum tissue; Mouse and rat cerebellum tissue. ICC/IF: Jurkat and HuT-78 cells. Flow Cyt (intra): Jurkat cells. mIHC: Human cerebellum tissue.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22040-80

Isotype

IgG

Applications

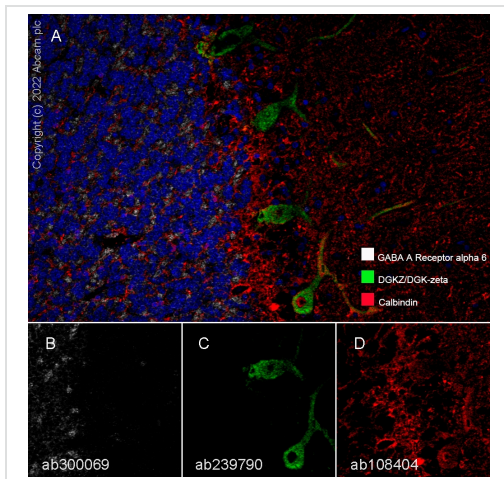
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab239081 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/600.
mIHC		Use at an assay dependent concentration.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 90-130 kDa (predicted molecular weight: 124 kDa).
ICC/IF		1/50.

Target

Function	Displays a strong preference for 1,2-diacylglycerols over 1,3-diacylglycerols, but lacks substrate specificity among molecular species of long chain diacylglycerols. Isoform 2 but not isoform 1 regulates RASGRP1 activity.
Tissue specificity	Highest levels in brain, and substantial levels in skeletal muscle, heart, and pancreas. Isoform 1 is predominantly expressed in muscle.
Sequence similarities	Belongs to the eukaryotic diacylglycerol kinase family. Contains 2 ANK repeats. Contains 1 DAGKc domain. Contains 2 phorbol-ester/DAG-type zinc fingers.
Post-translational modifications	Phosphorylation of the MARCKS homology domain by PKC reduces nuclear accumulation of DGK-zeta.
Cellular localization	Cytoplasm. Nucleus. Cell membrane.

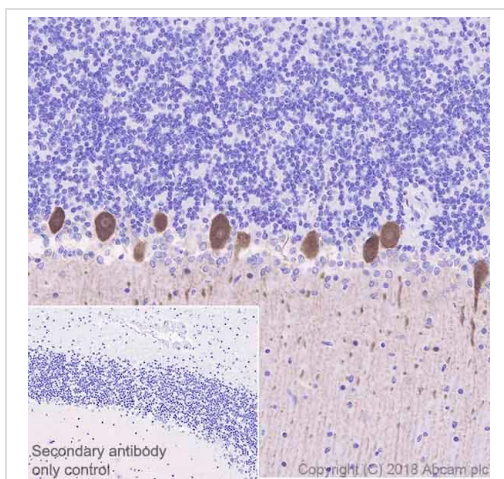
Images



Multiplex immunohistochemistry - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

Fluorescence multiplex immunohistochemical analysis of human cerebellum (formalin-fixed paraffin-embedded section). Merged staining of **ab300069**, GABA A Receptor alpha 6 at 1:500 (1.244 µg/ml) [Panel B], **ab239790**, DGKZ/DGK-zeta at 1:9000 (0.109 µg/ml) [Panel C], and **ab108404**, Calbindin at 1:1000 (1.394 µg/ml) [Panel D] on human cerebellum. Followed by Opal Polymer HRP Ms + Rb secondary. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. The section was incubated in three rounds of staining: in the order of **ab300069**, **ab239790**, and **ab108404** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. DAPI (dark blue) was used as a nuclear counter stain.

This data was developed using **ab239790**, the same antibody clone in a different buffer formulation.

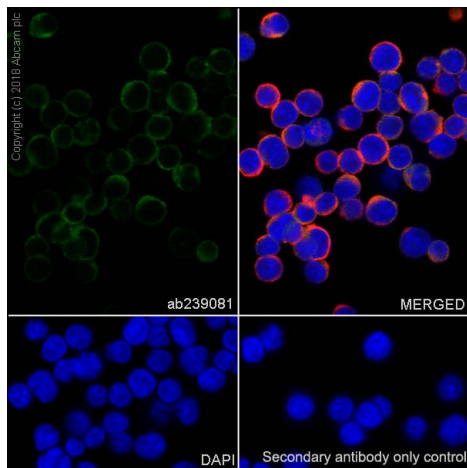


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

Immunohistochemical analysis of paraffin-embedded human cerebellum tissue labeling DGKZ/DGK-zeta with ab239081 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic and nuclear staining on Purkinje cells of human cerebellum (PMID: 14511325; PMID: 24119575) 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) Ready to use.

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



Immunocytochemistry/ Immunofluorescence - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

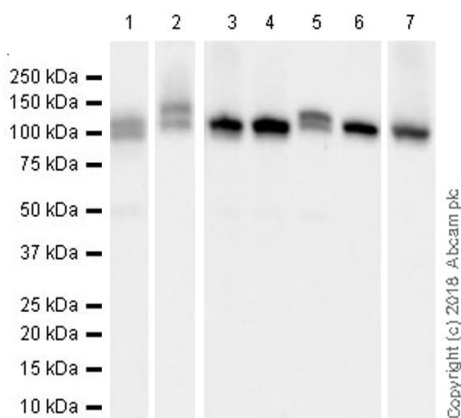
Immunofluorescent analysis of 100% methanol-fixed Jurkat (human T cell leukemia cell line from peripheral blood) cells labeling DGKZ/DGK-zeta with ab239081 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining in Jurkat cell line.

Methanol is recommended for fixation as weak signal was observed using 4% PFA.

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

All lanes : Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081) at 1/1000 dilution

Lane 1 : Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 2 : HuT-78 (human Sezary syndrome cutaneous T lymphocyte cell line) whole cell lysate

Lane 3 : Mouse brain lysate

Lane 4 : Mouse cerebellum lysate

Lane 5 : Mouse thymus lysate

Lane 6 : Rat brain lysate

Lane 7 : Rat cerebellum lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 124 kDa

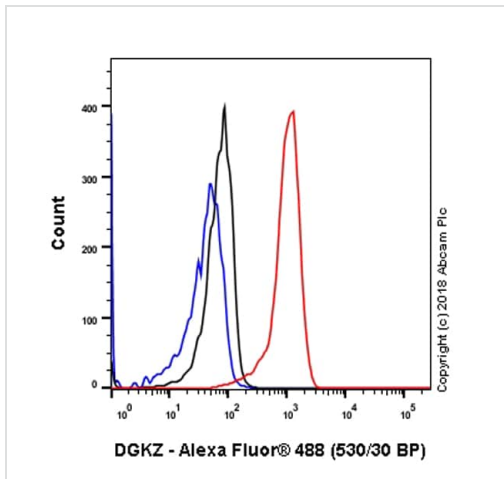
Observed band size: 90-130 kDa

Exposure time: Lane 1: 3 seconds; Lanes 2 & 7: 10 seconds; Lanes 3-6: 37 seconds.

Blocking/Dilution buffer: 5% NFD/MBST.

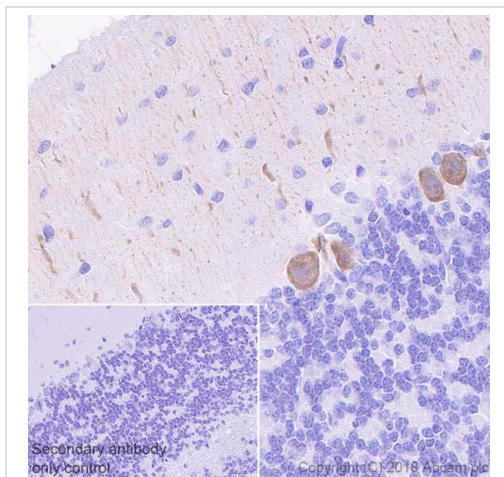
The lysates were not-boiled to avoid the protein aggregation.

Multiple bands observed are likely due to isoforms and phosphorylation. The molecular mass of human DGKZ / DGK-zeta is also predicted to be larger than that of rodents. The molecular profile/weight observed is consistent with what has been described in the literature (PMID: 12070163, PMID: 14511325, PMID: 9657393).



Flow Cytometry (Intracellular) - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized Jurkat (human T cell leukemia cell line from peripheral blood) cell line labeling DGKZ/DGK-zeta with ab239081 at 1/600 (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

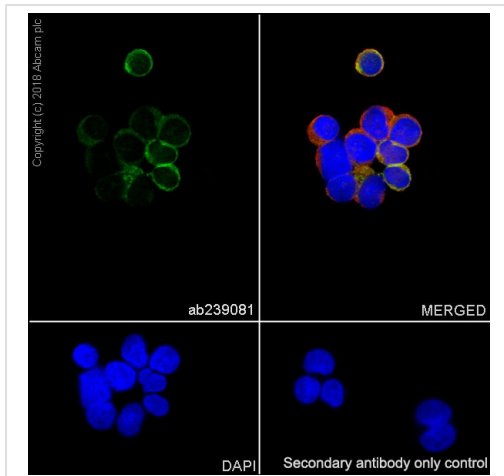


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue labeling DGKZ/DGK-zeta with ab239081 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic and nuclear staining on Purkinje cells of rat cerebellum (PMID: 14511325; PMID: 24119575) 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) Ready to use.

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



Immunocytochemistry/ Immunofluorescence - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

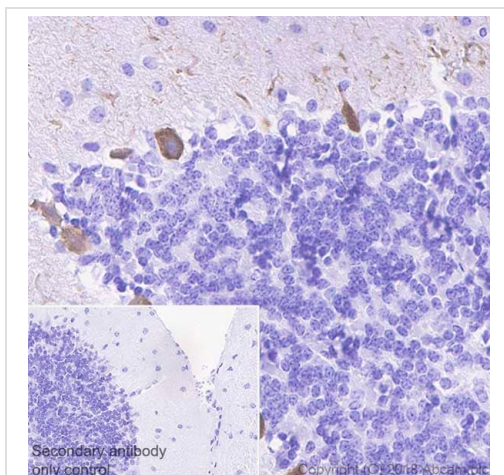
Immunofluorescent analysis of 100% methanol-fixed HuT-78 (human Sezary syndrome cutaneous T lymphocyte cell line) cells labeling DGKZ/DGK-zeta with ab239081 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining in HuT-78 cell line.

Methanol is recommended for fixation as weak signal was observed using 4% PFA.

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.

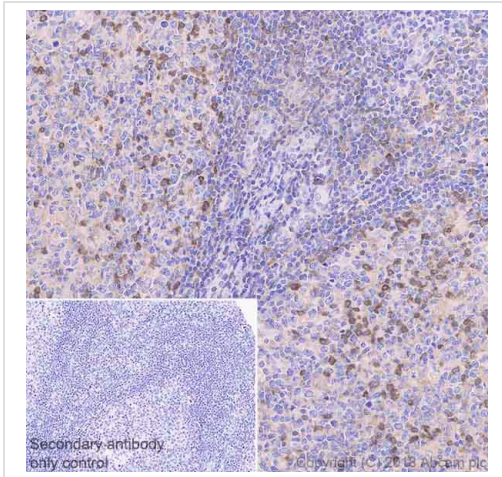


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue labeling DGKZ/DGK-zeta with ab239081 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic and nuclear staining on Purkinje cells of mouse cerebellum (PMID: 14511325; PMID: 24119575) 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) Ready to use.

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)





Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling DGKZ/DGK-zeta with ab239081 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on subset of immune cells in human tonsil (PMID: 24573202) 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) Ready to use.

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

Why choose a recombinant antibody?

 Research with confidence Consistent and reproducible results	 Long-term and scalable supply Recombinant technology
 Success from the first experiment Confirmed specificity	 Ethical standards compliant Animal-free production

Anti-DGKZ/DGK-zeta antibody [EPR22040-80] (ab239081)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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