# abcam

## Product datasheet

## Anti-Filamin A antibody [EP2405Y] ab76289





★★★★★ 6 Abreviews 36 References 8 Images

#### Overview

**Product name** Anti-Filamin A antibody [EP2405Y]

**Description** Rabbit monoclonal [EP2405Y] to Filamin A

**Host species** Rabbit

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

Unsuitable for: IP

Species reactivity Reacts with: Mouse, Rat, Human, African green monkey

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

Positive control COS-1, HeLa, HepG2, 3T3 and C6 cell lysate and human uterus tissue.

**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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

**Purity** Protein A purified

Clonality Monoclonal Clone number EP2405Y

ΙgG Isotype

## **Applications**

### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab76289 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		1/100.
WB	<b>★★★★ (4)</b>	1/250000 - 1/500000. Detects a band of approximately 281 kDa (predicted molecular weight: 281 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/100.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

#### **Application notes**

Is unsuitable for IP.

#### **Target**

## **Function**

Promotes orthogonal branching of actin filaments and links actin filaments to membrane glycoproteins. Anchors various transmembrane proteins to the actin cytoskeleton and serves as a scaffold for a wide range of cytoplasmic signaling proteins. Interaction with FLNA may allow neuroblast migration from the ventricular zone into the cortical plate. Tethers cell surface-localized furin, modulates its rate of internalization and directs its intracellular trafficking.

## Tissue specificity

#### Involvement in disease

## Ubiquitous.

Defects in FLNA are the cause of periventricular nodular heterotopia type 1 (PVNH1) [MIM:300049]; also called nodular heterotopia, bilateral periventricular (NHBP or BPNH). PVNH is a developmental disorder characterized by the presence of periventricular nodules of cerebral gray matter, resulting from a failure of neurons to migrate normally from the lateral ventricular proliferative zone, where they are formed, to the cerebral cortex. PVNH1 is an X-linked dominant form. Heterozygous females have normal intelligence but suffer from seizures and various manifestations outside the central nervous system, especially related to the vascular system. Hemizygous affected males die in the prenatal or perinatal period.

Defects in FLNA are the cause of periventricular nodular heterotopia type 4 (PVNH4) [MIM:300537]; also known as periventricular heterotopia Ehlers-Danlos variant. PVNH4 is characterized by nodular brain heterotopia, joint hypermobility and development of aortic dilation in early adulthood.

Defects in FLNA are the cause of otopalatodigital syndrome type 1 (OPD1) [MIM:311300]. OPD1 is an X-linked dominant multiple congenital anomalies disease mainly characterized by a generalized skeletal dysplasia, mild mental retardation, hearing loss, cleft palate, and typical facial anomalies. OPD1 belongs to a group of X-linked skeletal dysplasias known as oto-palatodigital syndrome spectrum disorders that also include OPD2, Melnick-Needles syndrome (MNS), and frontometaphyseal dysplasia (FMD). Remodeling of the cytoskeleton is central to the modulation of cell shape and migration. FLNA is a widely expressed protein that regulates re-

organization of the actin cytoskeleton by interacting with integrins, transmembrane receptor complexes and second messengers. Males with OPD1 have cleft palate, malformations of the ossicles causing deafness and milder bone and limb defects than those associated with OPD2. Obligate female carriers of mutations causing both OPD1 and OPD2 have variable (often milder) expression of a similar phenotypic spectrum.

Defects in FLNA are the cause of otopalatodigital syndrome type 2 (OPD2) [MIM:304120]; also known as cranioorodigital syndrome. OPD2 is a congenital bone disorder that is characterized by abnormally modeled, bowed bones, small or absent first digits and, more variably, cleft palate, posterior fossa brain anomalies, omphalocele and cardiac defects.

Defects in FLNA are the cause of frontometaphyseal dysplasia (FMD) [MIM:305620]. FMD is a congenital bone disease characterized by supraorbital hyperostosis, deafness and digital anomalies.

Defects in FLNA are the cause of Melnick-Needles syndrome (MNS) [MIM:309350]. MNS is a severe congenital bone disorder characterized by typical facies (exophthalmos, full cheeks, micrognathia and malalignment of teeth), flaring of the metaphyses of long bones, s-like curvature of bones of legs, irregular constrictions in the ribs, and sclerosis of base of skull.

Defects in FLNA are the cause of X-linked congenital idiopathic intestinal pseudoobstruction (CIIPX) [MIM:300048]. CIIPX is characterized by a severe abnormality of gastrointestinal motility due to primary qualitative defects of enteric ganglia and nerve fibers. Affected individuals manifest recurrent signs of intestinal obstruction in the absence of any mechanical lesion. Defects in FLNA are the cause of FG syndrome type 2 (FGS2) [MIM:300321]. FG syndrome (FGS) is an X-linked disorder characterized by mental retardation, relative macrocephaly, hypotonia and constipation.

Defects in FLNA are the cause of terminal osseous dysplasia (TOD) [MIM:300244]. A rare X-linked dominant male-lethal disease characterized by skeletal dysplasia of the limbs, pigmentary defects of the skin and recurrent digital fibroma during infancy. A significant phenotypic variability is observed in affected females.

Defects in FLNA are the cause of cardiac valvular dysplasia X-linked (CVDX) [MIM:314400]. A rare X-linked heart disease characterized by mitral and/or aortic valve regurgitation. The histologic features include fragmentation of collagenous bundles within the valve fibrosa and accumulation of proteoglycans, which produces excessive valve tissue leading to billowing of the valve leaflets.

#### Sequence similarities

Belongs to the filamin family.

Contains 1 actin-binding domain.

Contains 2 CH (calponin-homology) domains.

Contains 24 filamin repeats.

#### **Domain**

Comprised of a NH2-terminal actin-binding domain, 24 internally homologous repeats and two hinge regions. Repeat 24 and the second hinge domain are important for dimer formation.

# Post-translational modifications

 $Phosphory lated upon DNA \ damage, probably by ATM \ or \ ATR \ (By \ similarity). \ Phosphory lation$ 

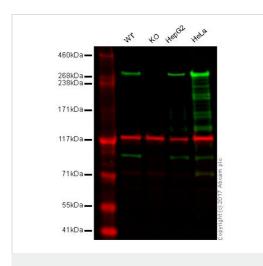
extent changes in response to cell activation.

The N-terminus is blocked.

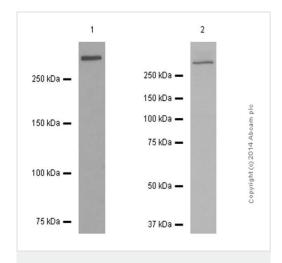
## **Cellular localization**

Cytoplasm > cell cortex. Cytoplasm > cytoskeleton.

### **Images**



Western blot - Anti-Filamin A antibody [EP2405Y] (ab76289)



Western blot - Anti-Filamin A antibody [EP2405Y] (ab76289)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

Lane 2: FLNA knockout HAP1 whole cell lysate (20 µg)

Lane 3: HepG2 whole cell lysate (20 μg)

Lane 4: HeLa whole cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab76289 observed at 281 kDa. Red - loading control, <u>ab18058</u>, observed at 130 kDa.

ab76289 was shown to specifically react with FLNA when FLNA knockout samples were used. Wild-type and FLNA knockout samples were subjected to SDS-PAGE. Ab76289 and <u>ab18058</u> (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 250000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

**All lanes**: Anti-Filamin A antibody [EP2405Y] (ab76289) at 1/10000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma) whole cell lysate

Lane 2 : COS-1 (Cercopithecus aethiops kidney) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

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

Predicted band size: 281 kDa

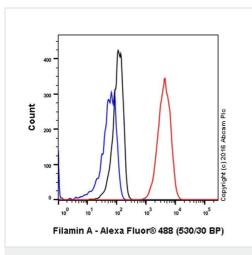
Additional bands at: 281 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 30 seconds

Blocking buffer: 5% NFDM/TBST

Diluting buffer: 5% NFDM/TBST

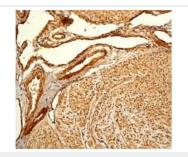


Flow Cytometry (Intracellular) - Anti-Filamin A antibody [EP2405Y] (ab76289)

ab76289 staining Filamin Ain the human cell line HeLa (human cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilized with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

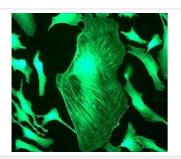
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Filamin A antibody
[EP2405Y] (ab76289)

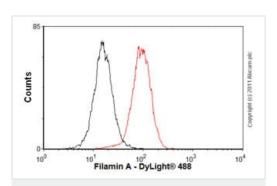
Immunohistochemical analysis of paraffin-embedded human uterus using ab76289 at a 1/100 dilution.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



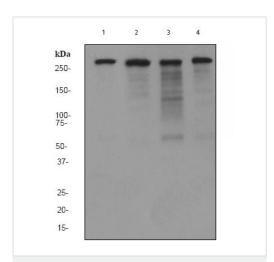
Immunocytochemistry/ Immunofluorescence - Anti-Filamin A antibody [EP2405Y] (ab76289)

Immunofluorescent staining of HeLa cells using ab76289 at a 1/100 dilution.



Flow Cytometry (Intracellular) - Anti-Filamin A antibody [EP2405Y] (ab76289)

Overlay histogram showing A431 cells stained with ab76289 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76289 , 1/100 dilutio) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1 $\mu$ g/1x106 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in A431 cells fixed with 4% paraformaldehyde/permeabilized in 0.1% PBS-Triton used under the same conditions.



Western blot - Anti-Filamin A antibody [EP2405Y] (ab76289)

**All lanes**: Anti-Filamin A antibody [EP2405Y] (ab76289) at 1/500000 dilution

Lane 1 : COS-1 (African green monkey kidney fibroblast-like cell line) cell lysate

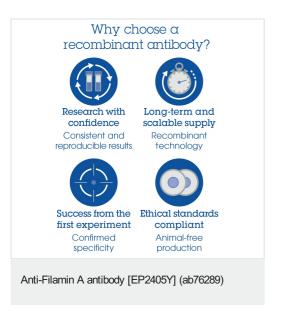
Lane 2 : Hela cell lysate
Lane 3 : 3T3 cell lysate
Lane 4 : C6 cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: HRP labelled goat anti-rabbit at 1/1000 dilution

Predicted band size: 281 kDa Observed band size: 281 kDa



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

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