# abcam

### Product datasheet

## Anti-ARF1 + ARF3 antibody [EP442Y] ab32524

Recombinant RabMAb

#### 7 References 9 Images

#### Overview

| Product name        | Anti-ARF1 + ARF3 antibody [EP442Y]  |  |
|---------------------|---|--|
| Description         | Rabbit monoclonal [EP442Y] to ARF1 + ARF3   |  |
| Host species        | Rabbit  |  |
| Specificity         | The immunogen used for this product shares 100% homology with ARF3, ARF4 and ARF5.<br>Cross-reactivity with the latter two protein have not been confirmed experimentally.  |  |
| Tested applications | Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF<br>Unsuitable for: IP   |  |
| Species reactivity  | Reacts with: Mouse, Rat, Human  |  |
| Immunogen           | Synthetic peptide within Human ARF1 aa 50-150. The exact sequence is proprietary.   |  |
| Positive control    | HEK-293 cell lysates and human breast carcinoma tissue.   |  |
| General notes       | <ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul> |  |

| Properties           |  |
|----------------------|--|
| Form                 | Liquid   |
| Storage instructions | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.                                |
| Storage buffer       | pH: 7.20<br>Preservative: 0.01% Sodium azide<br>Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA |
| Purity               | Protein A purified   |
| Clonality            | Monoclonal   |
| Clone number         | EP442Y   |

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab32524 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) |           | 1/80.<br><u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an<br>isotype control with this antibody.                                      |
| WB               |           | 1/2000. Detects a band of approximately 20 kDa (predicted molecular weight: 21 kDa).  |
| IHC-P            |           | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| ICC/IF           |           | 1/500.  |

**Application notes** 

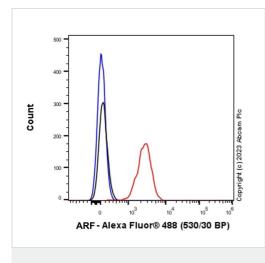
Is unsuitable for IP.

#### Target

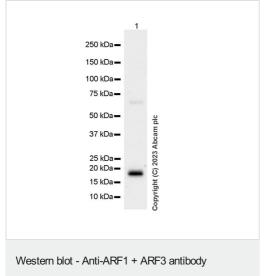
**Cellular localization** 

ARF1: Golgi apparatus. Cytoplasm > perinuclear region. ARF3: Golgi apparatus. Cytoplasm > perinuclear region.

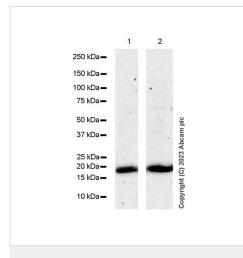
#### Images



Flow Cytometry - Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524) Flow cytometry analysis of 4% paraformaldehyde fixed 90% methanol permeabilised MCF7 (human breast adenocarcinoma epithelial cell) cells using ab32524 at 1/500 dilution (red) and secondary Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/5000 dilution. The isotype control was Rabbit monoclonal IgG (**ab172730**) (Black) and the control was cell swithout incubation with primary antibody and secondary antibody (Blue).



[EP442Y] (ab32524)



Western blot - Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524) Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524) at 1/1000 dilution + MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 21 kDa Observed band size: 20 kDa

Blocking and diluting buffer: 5% NFDM/TBST. Lysate was freshly made and used for Western blotting immediately to minimize protein degradation.

All lanes : Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524) at 1/1000 dilution

Lane 1 : SiHa (human cervical epithelial cell) whole cell lysate Lane 2 : T-47D (human ductal breast epithelial tumor epithelial cell) whole cell lysate

Lysates/proteins at 50 µg per lane.

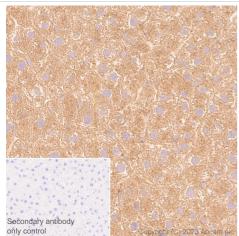
#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 21 kDa Observed band size: 20 kDa

Exposure time: 180 seconds

Blocking and diluting buffer: 5% NFDM/TBST

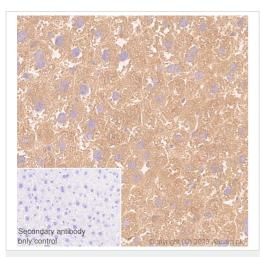


embedded sections) - Anti-ARF1 + ARF3 antibody

[EP442Y] (ab32524)

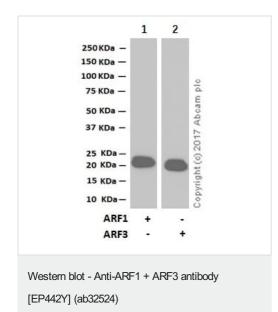
RX instru Secondary antibody only control Immunohistochemistry (Formalin/PFA-fixed paraffin-

Immunohistochemistry analysis of paraffin-embedded rat liver tissue sections using ab32524 at 1/2000 dilution and ready to use secondary LeicaDS9800 (Bond <sup>™</sup> Polymer Refine Detection). Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. Positive staining on rat liver. The section was incubated with ab32524 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524)

Immunohistochemistry analysis of paraffin-embedded mouse liver tissue sections using ab32524 at 1/2000 dilution and ready to use secondary LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection). Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. Positive staining on mouse liver. The section was incubated with ab32524 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



All lanes : Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524) at 1/20000 dilution

Lane 1 : Full length human ARF-1 recombinant protein Lane 2 : Full length human ARF-3 recombinant protein

Lysates/proteins at 0.015 µg per lane.

#### Secondary

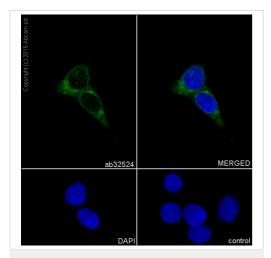
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 21 kDa Observed band size: 23 kDa

Exposure time: 3 minutes

Blocking and diluting buffer: 5% NFDM/TBST

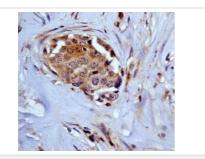
Human ARF-1 recombinant protein, (Cat#:<u>ab123538</u>) Human ARF-3 recombinant protein, (Cat#:<u>ab113581</u>)



Immunocytochemistry/ Immunofluorescence - Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524) Immunofluorescence staining of MCF7 cells with purified ab32524 working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor<sup>®</sup> 488 conjugated goat anti-rabbit (<u>ab150077</u>), used at a dilution of 1/1000. The cells were fixed in 100% methanol. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.

Immunohistochemical analysis of paraffin-embedded human breast carcinoma using ab32524 at 1/250 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524)



Anti-ARF1 + ARF3 antibody [EP442Y] (ab32524)

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