Overview

Product name  Anti-p57 Kip2 antibody [EP2515Y]
Description  Rabbit monoclonal [EP2515Y] to p57 Kip2
Host species  Rabbit
Tested applications  Suitable for: ICC/IF, WB, IP, IHC-P, Flow Cyt
Species reactivity  Reacts with: Mouse, Rat, Human
Immunogen  Synthetic peptide within Human p57 Kip2 aa 50-150 (N terminal). The exact sequence is proprietary.
General notes  Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

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, 0.05% BSA
Purity  Protein A purified
### Clonality
Monoclonal

### Clone number
EP2515Y

### Isotype
IgG

### Function
Potent tight-binding inhibitor of several G1 cyclin/CDK complexes (cyclin E-CDK2, cyclin D2-CDK4, and cyclin A-CDK2) and, to lesser extent, of the mitotic cyclin B-CDC2. Negative regulator of cell proliferation. May play a role in maintenance of the non-proliferative state throughout life.

### Tissue specificity
Expressed in the heart, brain, lung, skeletal muscle, kidney, pancreas and testis. High levels are seen in the placenta while low levels are seen in the liver.

### Involvement in disease
Defects in CDKN1C are a cause of Beckwith-Wiedemann syndrome (BWS) [MIM:130650]. BWS is a genetically heterogeneous disorder characterized by anterior abdominal wall defects including exomphalos (omphalocele), pre- and postnatal overgrowth, and macroglossia. Additional less frequent complications include specific developmental defects and a predisposition to embryonal tumors.

Note=Defects in CDKN1C are involved in tumor formation.

### Sequence similarities
Belongs to the CDI family.

### Cellular localization
Nucleus.

### Applications

Our [Abpromise guarantee](#) covers the use of ab75974 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/250.</td>
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<tr>
<td>IP</td>
<td></td>
<td>1/30.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See <a href="#">IHC antigen retrieval protocols</a>.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/350. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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### Target

#### Function
Potent tight-binding inhibitor of several G1 cyclin/CDK complexes (cyclin E-CDK2, cyclin D2-CDK4, and cyclin A-CDK2) and, to lesser extent, of the mitotic cyclin B-CDC2. Negative regulator of cell proliferation. May play a role in maintenance of the non-proliferative state throughout life.

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### Images
Western blot - Anti-p57 Kip2 antibody [EP2515Y] (ab75974)

All lanes: Anti-p57 Kip2 antibody [EP2515Y] (ab75974) at 1/500 dilution (purified)

Lane 1: Mouse brain
Lane 2: Rat brain

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 32 kDa
Observed band size: 57 kDa

why is the actual band size different from the predicted?

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling p57 Kip2 with purified ab75974 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/250) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human placenta tissue labelling p57 Kip2 with purified ab75974 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Flow cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling p57 Kip2 (red) with ab75974 at a 1/350 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (ab172730). Blue (unlabeled control) - Cells without incubation with primary and secondary antibodies.
ab75974 (purified) at 1/30 immunoprecipitating p57 Kip2 in HeLa cells treated with dexamethasone (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

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Anti-p57 Kip2 antibody [EP2515Y] (ab75974) at 1/500 dilution (purified) + Human brain at 20 µg

**Secondary**

HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG at 1/1000 dilution

**Predicted band size:** 32 kDa

**Observed band size:** 57 kDa

*why is the actual band size different from the predicted?*

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.
Western blot - Anti-p57 Kip2 antibody [EP2515Y] (ab75974)

All lanes: Anti-p57 Kip2 antibody [EP2515Y] (ab75974) at 1/500 dilution (unpurified)

Lane 1: HeLa lysate
Lane 2: Jurkat lysate
Lane 3: Human brain lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 32 kDa
Observed band size: 57 kDa why is the actual band size different from the predicted?

Immunocytochemistry/Immunofluorescence - Anti-p57 Kip2 antibody [EP2515Y] (ab75974)

ab75974 staining p57 Kip2 in HeLa cells treated with dexamethasone (ab120743), by ICC/IF. Increase in p57 Kip2 expression correlates with increased concentration of dexamethasone as described in literature.

The cells were incubated at 37°C for 24h in media containing different concentrations of ab120743 (dexamethasone) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with unpurified ab75974 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

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