

# Anti-NDP52/CALCOCO2 Antibody Picoband<sup>™</sup> (monoclonal, 9E2F2)

Catalog # ABO16602

# Specification

# Anti-NDP52/CALCOCO2 Antibody Picoband<sup>™</sup> (monoclonal, 9E2F2) - Product Information

Application	WB, IF, ICC, FC
Primary Accession	<u>Q13137</u>
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	
Anti-NDP52/CALCOCO2 Antibody Picoband™ (me	onoclonal, 9E2F2) . Tested in Flow Cytometry, IF,
ICC, WB applications. This antibody reacts with I	Human, Mouse, Rat.

Reconstitution Adding 0.2 ml of distilled water will yield a concentration of 500  $\mu$ g/ml.

# Anti-NDP52/CALCOCO2 Antibody Picoband<sup>™</sup> (monoclonal, 9E2F2) - Additional Information

Gene ID 10241

**Other Names** Calcium-binding and coiled-coil domain-containing protein 2, Antigen nuclear dot 52 kDa protein, Nuclear domain 10 protein NDP52, Nuclear domain 10 protein 52, Nuclear dot protein 52, CALCOCO2, NDP52 {ECO:0000303|PubMed:7540613}

Calculated MW 52 kDa KDa

**Application Details** 

Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat<br>
Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human<br>> Flow Cytometry, 1-3 µg/1x10^6 cells, Human<br>>

**Contents** Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen E.coli-derived human NDP52/CALCOCO2 recombinant protein (Position: M1-L446).

**Purification** Immunogen affinity purified.

Storage

At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen



at -20°C for six months. Avoid repeated freezing and thawing.

# Anti-NDP52/CALCOCO2 Antibody Picoband™ (monoclonal, 9E2F2) - Protein Information

Name CALCOCO2

Synonyms NDP52 {ECO:0000303|PubMed:7540613}

#### Function

Xenophagy-specific receptor required for autophagy-mediated intracellular bacteria degradation. Acts as an effector protein of galectin-sensed membrane damage that restricts the proliferation of infecting pathogens such as Salmonella typhimurium upon entry into the cytosol by targeting LGALS8-associated bacteria for autophagy (PubMed:<a

href="http://www.uniprot.org/citations/22246324" target="\_blank">22246324</a>). Initially orchestrates bacteria targeting to autophagosomes and subsequently ensures pathogen degradation by regulating pathogen-containing autophagosome maturation (PubMed:<a href="http://www.uniprot.org/citations/23022382" target="\_blank">23022382</a>, PubMed:<a href="http://www.uniprot.org/citations/25771791" target="\_blank">25771791</a>). Bacteria targeting to autophagosomes relies on its interaction with MAP1LC3A, MAP1LC3B and/or GABARAPL2, whereas regulation of pathogen-containing autophagosome maturation requires the interaction with MAP3LC3C (PubMed:<a href="http://www.uniprot.org/citations/23022382" target="\_blank">23022382</a>, PubMed:<a href="http://www.uniprot.org/citations/25771791" target="\_blank">23022382</a>, PubMed:<a href="http://www.uniprot.org/citations/25771791" target="\_blank">25771791</a>). May play a role in ruffle formation and actin cytoskeleton organization and seems to negatively regulate constitutive secretion (PubMed:<a href="http://www.uniprot.org/citations/17635994" target="\_blank">17635994</a>).

#### **Cellular Location**

Cytoplasm, perinuclear region. Cytoplasm, cytoskeleton. Cytoplasmic vesicle, autophagosome membrane; Peripheral membrane protein. Note=According to PubMed:7540613, localizes to nuclear dots. According to PubMed:9230084 and PubMed:12869526, it is not a nuclear dot-associated protein but localizes predominantly in the cytoplasm with a coarse-grained distribution preferentially close to the nucleus.

#### **Tissue Location**

Expressed in all tissues tested with highest expression in skeletal muscle and lowest in brain

# Anti-NDP52/CALCOCO2 Antibody Picoband<sup>™</sup> (monoclonal, 9E2F2) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- <u>Dot Blot</u>
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

# Anti-NDP52/CALCOCO2 Antibody Picoband<sup>™</sup> (monoclonal, 9E2F2) - Images





Figure 1. Western blot analysis of NDP52/CALCOCO2 using anti-NDP52/CALCOCO2 antibody (M05876-2).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human placenta tissue lysates,

Lane 2: human Hela whole cell lysates,

Lane 3: human Jurkat whole cell lysates,

Lane 4: human A549 whole cell lysates,

Lane 5: rat testis tissue lysates,

Lane 6: mouse testis tissue lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-NDP52/CALCOCO2 antigen affinity purified monoclonal antibody (Catalog # M05876-2) at 0.5  $\mu$ g/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for NDP52/CALCOCO2 at approximately 52 kDa. The expected band size for NDP52/CALCOCO2 is at 52 kDa.



Figure 2. IF analysis of NDP52/CALCOCO2 using anti-NDP52/CALCOCO2 antibody (M05876-2). NDP52/CALCOCO2 was detected in an immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 μg/mL mouse



anti-NDP52/CALCOCO2 Antibody (M05876-2) overnight at 4°C. Cy3 Conjugated Goat Anti-Mouse IgG (BA1031) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Figure 3. Flow Cytometry analysis of MCF-7 cells using anti-NDP52/CALCOCO2 antibody (M05876-2).

Overlay histogram showing MCF-7 cells stained with M05876-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-NDP52/CALCOCO2 Antibody (M05876-2, 1  $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10  $\mu$ g/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu$ g/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

# Anti-NDP52/CALCOCO2 Antibody Picoband™ (monoclonal, 9E2F2) - Background

This gene encodes a coiled-coil domain-containing protein. The encoded protein functions as a receptor for ubiquitin-coated bacteria and plays an important role in innate immunity by mediating macroautophagy. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene.