

Anti-POR Antibody Picoband™ (monoclonal, 7F5)

Catalog # ABO15083

Product Information

Application	WB, IHC, FC
Primary Accession	P16435
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-POR Antibody Picoband™ (monoclonal, 7F5) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	5447
Other Names	NADPH--cytochrome P450 reductase {ECO:0000255 HAMAP-Rule:MF_03212}, CPR {ECO:0000255 HAMAP-Rule:MF_03212}, P450R {ECO:0000255 HAMAP-Rule:MF_03212}, 1.6.2.4 {ECO:0000255 HAMAP-Rule:MF_03212}, POR {ECO:0000255 HAMAP-Rule:MF_03212}, CYPOR
Calculated MW	76690
Application Details	Western blot, 0.25-0.5 µg/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na ₂ HPO ₄ .
Clone Names	Clone: 7F5
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human POR, different from the related mouse and rat sequences by five amino acids.
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.

Protein Information

Name	POR {ECO:0000255 HAMAP-Rule:MF_03212}
Synonyms	CYPOR
Function	This enzyme is required for electron transfer from NADP to cytochrome P450 in microsomes. It can also provide electron transfer to heme oxygenase and cytochrome B5.
Cellular Location	Endoplasmic reticulum membrane {ECO:0000255 HAMAP-Rule:MF_03212}; Single-pass membrane protein {ECO:0000255 HAMAP-Rule:MF_03212}; Cytoplasmic side {ECO:0000255 HAMAP-Rule:MF_03212}

Background

POR is a membrane-bound enzyme required for electron transfer from NADPH to cytochrome P450 in the endoplasmic reticulum of the eukaryotic cell. The gene encodes an endoplasmic reticulum membrane oxidoreductase with an FAD-binding domain and a flavodoxin-like domain. The protein binds two cofactors, FAD and FMN, which allow it to donate electrons directly from NADPH to all microsomal P450 enzymes. Mutations in this gene have been associated with various diseases, including apparent combined P450C17 and P450C21 deficiency, amenorrhea and disordered steroidogenesis, congenital adrenal hyperplasia and Antley-Bixler syndrome.

Images

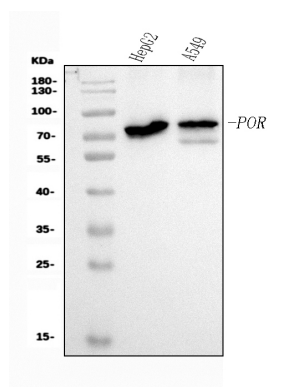


Figure 1. Western blot analysis of POR using anti-POR antibody (M02166-2).

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 μ g of sample under reducing conditions.

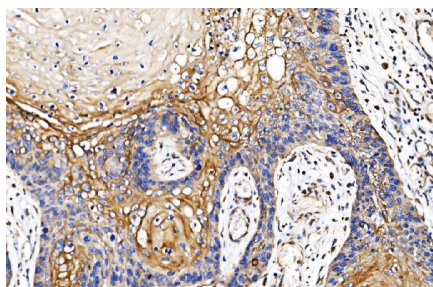
Lane 1: human HepG2 whole cell lysates,

Lane 2: human A549 whole cell 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-POR antigen affinity purified monoclonal antibody (Catalog # M02166-2) at 0.5 μ 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 POR at approximately 77 kDa. The expected band size for POR is at 77 kDa.

Figure 2. IHC analysis of POR using anti-POR antibody (M02166-2).

POR was detected in a paraffin-embedded section of human esophageal squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml mouse anti-POR Antibody



(M02166-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

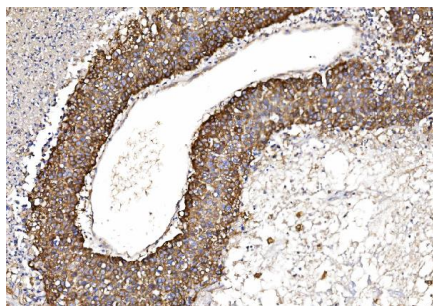


Figure 3. IHC analysis of POR using anti-POR antibody (M02166-2). POR was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-POR Antibody (M02166-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

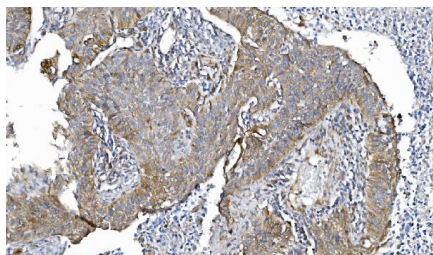


Figure 4. IHC analysis of POR using anti-POR antibody (M02166-2). POR was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-POR Antibody (M02166-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

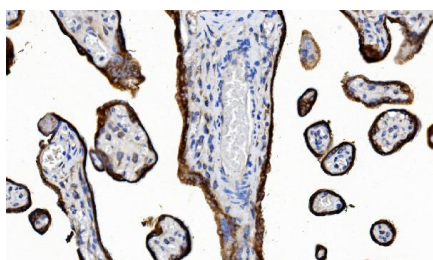
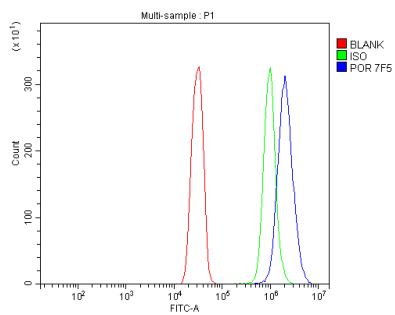


Figure 5. IHC analysis of POR using anti-POR antibody (M02166-2). POR was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-POR Antibody (M02166-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 6. Flow Cytometry analysis of SiHa cells using anti-POR antibody (M02166-2). Overlay histogram showing SiHa cells stained with M02166-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-POR Antibody (M02166-2, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary



antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG ($1 \mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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