# Anti-MRP1 Antibody [JA93-03]

### ET1704-56



Product Type: Species reactivity: Applications: Molecular Wt: Clone number:	Recombinant Rabbit monoclonal IgG, primary antibodies Human WB, IHC-P, FC, IF-Cell Predicted band size: 172 kDa JA93-03
Description:	The two members of the large family of ABC transporters known to confer multidrug resistance in human cancer cells are the Mdr-1 P-glycoprotein and the multidrug-resistance protein MRP1. MRP1 is an integral membrane protein that contains an Mdr-like core, an N-terminal membrane-bound region and a cytoplasmic linker, and it is expressed in various cerebral cells, as well as in lung, testis and peripheral blood. The MRP gene family also includes MRP2, which is alternatively designated cMOAT (for canalicular multispecific organic anion transporter) and MRP3, which are both conjugate export pumps expressed predominantly in hepatocytes. MRP2 localizes exclusively to the apical membrane and is constitutively expressed at a high level in normal liver cells. Conversely, MRP3 localizes to the basolateral membrane where it also mediates the transport of the organic anion S-(2,4-dinitrophenyl-) glutathione toward the basolateral side of the membrane. MRP3 is normally expressed at comparatively lower levels than MRP2 and increases only when secretion across the apical membrane by MRP2 is impaired. MRP6 protein is highly expressed in liver and kidney, whereas MRP4 and MRP5 are detected in various tissues yet at much lower levels of expression.
lmmunogen:	Synthetic peptide within Human MRP1 aa 1482-1531 / 1531.
Positive control:	A549 cell lysate, HepG2 cell lysate, A549, human pancreas tissue, human tonsil tissue.
Subcellular location:	Cell membrane.
Database links:	SwissProt: P33527 Human
Recommended Dilutions: WB IHC-P FC IF-Cell	1:1,000 1:50-1:200 1:1,000 1:100
Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!{\rm C}$ after thawing. Aliquot store at -20 $^\circ\!{\rm C}$ or -80 $^\circ\!{\rm C}$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

## Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

#### Images

kDa\_A5A9

220kDa

MRP1

HSP90 + - heat

250

150

100 72

> 55 45 35

25

14

Fig1: Western blot analysis of MRP1 on different lysates with Rabbit anti-MRP1 antibody (ET1704-56) at 1/1,000 dilution.

Lane 1: A549 cell lysate Lane 2: A549 cell lysate (no heat) Lane 3: HepG2 cell lysate Lane 4: HepG2 cell lysate (no heat)

Notice: no heat means the lysate is not boiled.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 172 kDa Observed band size: 220 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-56) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of MRP1 on different lysates with Rabbit anti-MRP1 antibody (ET1704-56) at 1/1,000 dilution.

Lane 1: A549-si NT cell lysate (no heat) Lane 2: A549-si MRP1 cell lysate (no heat)

Notice: no heat means the lysate is not boiled.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 172 kDa Observed band size: 220 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-56) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature

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**Fig3:** Immunocytochemistry analysis of A549 cells labeling MRP1 with Rabbit anti-MRP1 antibody (ET1704-56) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-MRP1 antibody (ET1704-56) at 1/100 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor<sup>TM</sup> 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 $^{\circ}$ C. Goat Anti-Goat Anti-Mouse IgG H&L (iFluor<sup>TM</sup> 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

**Fig4:** Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-MRP1 antibody. The section was pretreated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1704-56, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

**Fig5:** Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-MRP1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1704-56, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



Fig6: Flow cytometric analysis of A549 cells labeling MRP1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1704-56, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Lin H. et. al. KDM5c inhibits multidrug resistance of colon cancer cell line by down-regulating ABCC1. Biomed Pharmacother. 2018 Nov
- 2. Li S. et. al. Down-regulation of miR-210-3p encourages chemotherapy resistance of renal cell carcinoma via modulating ABCC1. Cell Biosci. 2018 Feb

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