

Basic Information

Product Name	Anti-BCRP/ABCG2 Antibody	
Gene Name	ABCG2	
Source	Rabbit	
Isotype	IgG	
Species Reactivity	human, mouse, rat	
Tested Application	WB, IHC, IHC-F, ICC, FCM	
Contents	500 ug/ml antibody with PBS , 0.02% NaN ₃ , 1 mg BSA and 50% glycerol.	
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human ABCG2(137-168aa RENLQFSAALRLATTMTNHEKNERINRVIQEL), different from the related mouse sequence by five amino acids, and from the related rat sequence by eight amino acids.	
concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	65-80KD	
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry in paraffin section (IHC): 1:50-400 Immunohistochemistry in frozen section (IHC-F): 1:50-400 Immunocytochemistry: 1:50-400 Flow cytometry (FCM): 1-3 µg/1x10 ⁶ cells (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

Background Information

ABCG2(Atp-binding cassette, subfamily g, member 2) also known as ABCP, BCRP or MRX, is a protein that in humans is encoded by the ABCG2 gene. The ABCG2 gene encodes a membrane transporter belonging to the ATP-binding cassette (ABC) superfamily of membrane transporters, which are involved in the trafficking of biologic molecules across cell membranes. The ABCG2 protein is also a high capacity transporter for uric acid excretion in the kidney, liver, and gut. The ABCG2 gene is mapped on 4q22.1. In vitro assays of isolated membrane preparations revealed a high-capacity, vanadate-sensitive ATPase activity associated with ABCG2 expression that was stimulated by compounds known to be transported by this protein. Ozvegy et al. (2001) concluded that ABCG2 is likely functioning as a homodimer or homooligomer in this expression system since it is unlikely that putative Sf9 transport partners would be overexpressed at similarly high levels. Abcg2 transports pheophorbide-a, which occurs in various plant-derived foods and food supplements and is highly

efficient in limiting its uptake from ingested food. ABCG2 is a major factor in the concentrative transfer of drugs, carcinogens, and dietary toxins to the milk of mice, cows, and humans.

Selected Validation Data



Figure 1. Western blot analysis of ABCG2 using anti-ABCG2 antibody (PB9364). 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 50ug of sample under reducing conditions. Lane 1: Human Placenta Tissue Lysate, Lane 2: HELA Whole Cell Lysate, Lane 3: PANC Whole Cell Lysate, Lane 4: COLO320 Whole Cell Lysate. After electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ABCG2 antigen affinity purified polyclonal antibody (Catalog # PB9364) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for ABCG2 at approximately 72KD. The expected band size for ABCG2 is at 72KD.

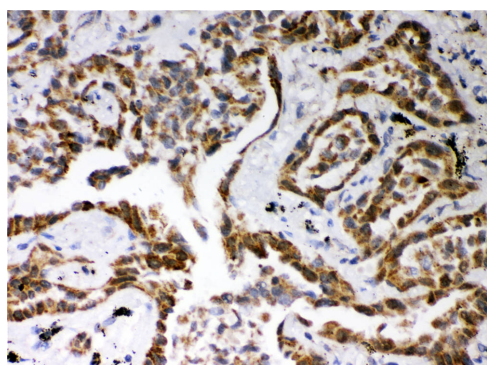


Figure 2. IHC analysis of ABCG2 using anti-ABCG2 antibody (PB9364). ABCG2 was detected in paraffin-embedded section of Human Lung Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1µg/ml rabbit anti-ABCG2 Antibody (PB9364) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

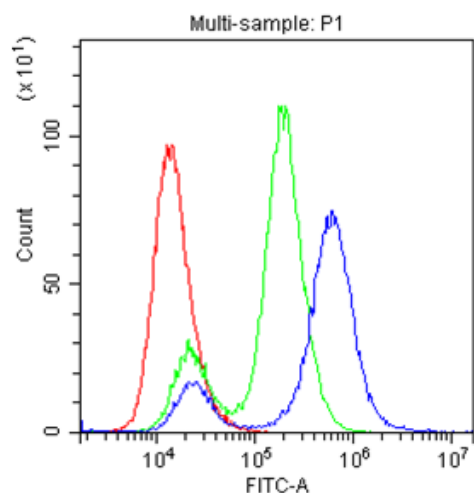


Figure 5. Flow Cytometry analysis of U-87MG cells using anti-ABCG2 antibody (PB9364). Overlay histogram showing U-87MG cells stained with PB9364 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ABCG2 Antibody (PB9364, 1 μ g/1 \times 10⁶ cells) for 30 min at 20°C. DyLight488 conjugated goat anti-rabbit IgG (BA1127, 5-10 μ g/1 \times 10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 μ g/1 \times 10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.