RABBIT ANTI-HERG (hK_V11.1) AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5908-200UL

LOT NUMBER:

QUANTITY: 200 μ L

CONCENTRATION: 0.6 mg/mL (after reconstitution)

SPECIFICITY: Recognizes HERG (hK_v11.1, Voltage gated K+ channel subfamily H

member 2, KCNH2, ether-a-go-go related channel). The antibody recognizes the HERG1b splice variant but not splice variants HERG1-3 or HERG-4. The immunogen shares homology with rat erg2 (22/54)

and rat erg3 (21/54).

IMMUNOGEN: GST fusion protein corresponding to amino acids 1106-1159 of human

erg1 (HERG) (Accession number Q12809).

APPLICATIONS: Western blot: 1:200-1:400 using ECL on HERG transfected HEK293

cells.

Immunocytochemistry Immunprecipitation

Dilutions should be made using a carrier protein such as BSA (1-3%) Optimal working dilutions must be determined by the end user.

CONTROL ANTIGEN: Included free of charge with the antibody is XX μg of fusion protein (35

kD). The stock solution of the antigen can be made up using 100 μ L of PBS. For negative control, preincubate 3 μ g of fusion protein with 1 μ g of antibody for one hour at room temperature. For positive control, use 20 ng of fusion protein per lane. Optimal concentrations must be

determined by the end user.

SPECIES REACTIVITIES: Human. It has also been reported that the antibody will recognize

mouse and horse. Other species have not been tested. It is expected that the antibody may also react with rabbit (identical), canine (51/54),

mouse (50/54) and rat (50/54) due to sequence homology.

FORMAT: Affinity purified immunoglobulin.

PRESENTATION: Lyophilized from phosphate buffered saline, pH 7.4, containing 1%

BSA, and 0.025% sodium azide as a preservative. Reconstitute with 200 μL of sterile deionized water. Centrifuge antibody preparation

before use (10,000 xg for 5 min).

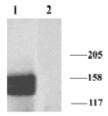
STORAGE/HANDLING: Maintain lyophilized material at -20°C for up to 12 months after date of

receipt. After reconstitution maintain at -20°C in undiluted aliquots for

up to 6 months. Avoid repeated freeze/thaw cycles.

SUGGESTED WESTERN BLOT PROTOCOL

- 1. Mix the samples (organ membranes: 50 μ g/lane; transfected cells: 500,000 cells/lane) with sample-buffer X 2, and heat 10 min at 70°C.
- 5-50 μL applied to Minigel lane (0.75-1.5 mm width) and run at standard conditions. (60 mA for 2 1.5 mm Minigel gels, 1.4 h). It is suggested that you run 5-15% acrylamide (37.5:1 acrylamide:bisacrysmide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
- 3. Transfer in semi-dry system under standard conditions (3 h 100 mA for two minigel gels)
- 4. Stain the transferred bands with Millipore BLOT-FastStain (Catalog Number 2076).
- 5. Destain with deionized water.
- 6. Block with 5% non-fat milk (Marvel or Carnation) in PBS, and 0.025 % sodium azide, overnight at 2-8°C. The non-fat milk should be dissolved freshly, centrifuged 10,000 rpm for 10 min, and filtered through glass filter (Gelman Acrodisc).
- 7. Incubation with first antibody 2 h at room temperature or overnight at 4°C in blocking solution. The antibody preparation should be centrifuged before use (10,000 g 5 min.). Optimal working dilutions and incubation time will need to be determined by the end user.
- 8. Wash 4 x 10 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
- 9. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Millipore Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
- 10. Wash 4 x 10 min. with PBS-0.1% tween 20.
- 11. Perform ECL with commercial kits (Chemilucent, Millipore Catalog Number 2600).



Western blotting of lysate from HEK293 cells expressing HERG

- 1. AB5908 1:200
- 2. AB5908 preincubated with control fusion protein.

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