

RBHERGM: hERG K⁺ Channel (Cloned human ether-a-gogo related gene 1 potassium channel)

SPECIFIC LOT DATA: Lot 370-770-A

Receptor Concentration (B_{max}): 0.30 pmol/mg protein

K_d for [¹²⁵I]-BeKm-1 binding: 0.050 nM

Volume: 0.4 ml per 400 microassay size vial

Protein Concentration: 4.9 mg/ml

PACKAGING: Membranes suspended in 50 mM Tris-HCl (pH 7.4), 10% sucrose.

RECOMMENDED ASSAY PROCEDURE:

Thawing: Thaw vials rapidly; dilute with incubation buffer; homogenize. Keep on ice.

Incubation buffer: 20 mM Hepes-Tris, pH 7.2, 100 μ M KCl and 0.1% BSA.

Note: To prepare incubation buffer, use non pH-adjusted Hepes at a final concentration of 20 mM, add KCl and BSA and then adjust to pH 7.2 with a few drops of 1 M non pH-adjusted TRIS.

Binding protocol: Incubate 4.9 μ g of membranes (150 μ l of a 1:150 dilution in incubation buffer), buffer and [¹²⁵I]-BeKm-1 in a total volume of 200 μ l.

Add the assay components in the following order:

- 25 μ l 1 μ M BeKm-1 (0.125 μ M final; for non-specific binding);
or 25 μ l incubation buffer (for total binding);
- 25 μ l [¹²⁵I]-BeKm-1 at the appropriate concentration (see graphs on reverse);
- 150 μ l diluted membranes.

Incubate 60 min at 27°C.

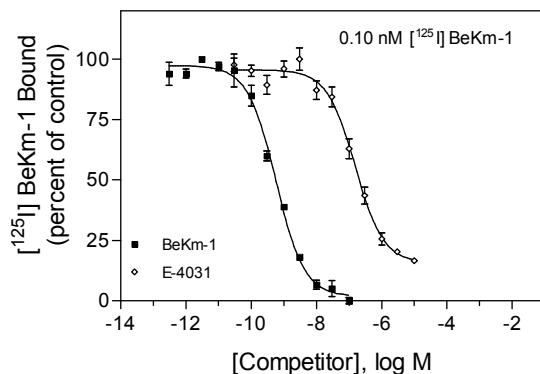
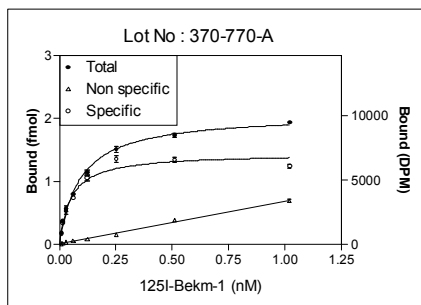
Filter over GF/C filter (presoaked in 0.3% polyethylenimine) and wash filter 9 \times with 0.5 ml ice-cold 20 mM Tris-HCl, pH 7.3 @ 4°C and 150 mM NaCl.

STABILITY AND STORAGE RECOMMENDATIONS: Storage at -80°C is recommended. Long term stability is currently under investigation. Repeated freeze-thawing of this product is not recommended.

ORIGIN: Human ERG1 K⁺ Channel expressed in HEK-293 cells.

- REFERENCES:**
- GenBank accession number: NM_000238 (KCNH2, transcript variant 1)
 - Trudeau, M.C. *et al.*, Science **269**: 92-95 (1995)
 - Angelo K. *et al.*, Pflugers Arch. **447**: 55-63 (2003)
 - Chiu *et al.*, J. Pharmacol. Sci. **95**: 311-319 (2004)
 - Finlayson *et al.*, Eur. J. Pharmacol. **430**: 147-148 (2001)

[¹²⁵I]-BeKm-1 binding to hERG K⁺ Channel



Affinity Constants for hERG K⁺ Channel Lot 370-770-A

COMPETITOR*	K _i (nM)
BeKm-1	0.18
E-4031	47

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

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