hERG K^+ Channel Membranes

For fast, robust & sensitive hERG safety screening

Assess potential cardiotoxicity of compounds early during the drug development cycle with a high-throughput hERG binding assay. The literature indicates that binding assay results for hERG strongly correlate with electrophysiological data.[1-3]

hERG binding assays using PerkinElmer's **hERG K^+ Channel** membranes are an ideal complement to whole cell patch clamp for the detection and initial characterization of hERG channel blockers. hERG K^+ Channel membranes show excellent expression and performance when tested with different radiolabeled hERG blockers, both in filtration and homogenous binding assays.

Perform faster, easier safety tests using PerkinElmer's hERG K^+ Channel membranes

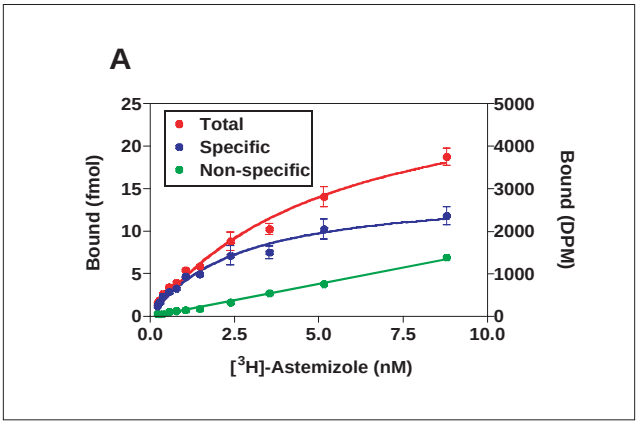
- **Easy to automate** – simple binding assay set-up
- **High expression** – membranes derived from a hERG1 HEK-293 cell line with B_{max} value for [^{3}H]-astemizole between 5-10 pmol/mg protein
- **Precise** – Z' of 0.7 obtained in a FlashBlue™ GPCR homogeneous binding assay with [^{125}I]-BeKm-1
- **HTS-compatible** – for 96- and 384-well formats
- **Choice of radioligands** – chose between PerkinElmer's [^{3}H]-astemizole and [^{125}I]-BeKm-1 radioligands
- **Read on any scintillation counter**, such as PerkinElmer's MicroBeta® or TopCount®

Radioligands for hERG binding assays

PerkinElmer offers two complementary radioligands for hERG binding assays that bind preferentially to distinct conformations of the hERG channel. PerkinElmer can also custom label your proprietary hERG blocker.

Astemizole, [O-Methyl-^{3}H]-: This small molecule binds to *open/inactivated* hERG K^+ channels. Typical binding assay results obtained with tritiated astemizole are illustrated in Figure 1. The pharmacology of [^{3}H]-astemizole binding has been shown to correlate well with patch clamp electrophysiological measurements[1], and with the binding of another radioligand, [^{3}H]-dofetilide[2].

hERG K^+ Channel binding assays with [^{3}H]-astemizole



Compound	K_i (nM)
Astemizole	18
Dofetilide	33
E-4031	134
Terfenadine	445.5
Quinidine	40.000

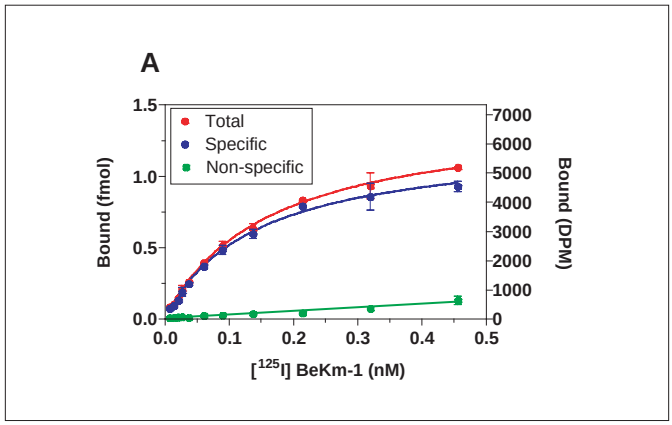
Figure 1A. [^{3}H]-Astemizole saturation binding assay performed using hERG K^+ Channel membranes. B_{max} value of 6 pmol/mg and K_d value of 3 nM were obtained.

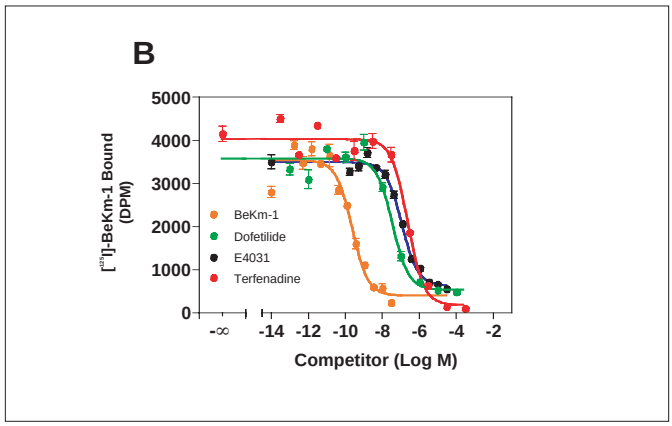
Figure 1B. In competition experiments, the radioligand was used at the K_d concentration. K_i values are the average of two independent experiments. A quantity of 2.5 µg of hERG K^+ Channel membranes was used per well. The assay was incubated for 1 h at room temperature. Signal was detected with a MicroBeta after filtration of the samples.

PerkinElmer® precisely.

BeKm-1, [^{125}I-Tyr^{11}]-: This peptidic toxin from the scorpion *Buthus eupeus* binds principally to *closed* hERG K^+ channels with an affinity in the sub-nanomolar range[4]. Results from filtration and homogeneous hERG binding assays obtained using iodinated BeKm-1 are illustrated in Figures 2 and 3, respectively.

hERG K^+ Channel binding assays with [^{125}I]-BeKm-1





Compound	K_i (nM)
BeKm-1	0.13
Dofetilide	23
E-4031	61
Terfenadine	174

Figure 2A. [^{125}I]-BeKm-1 saturation binding assay performed using hERG K^+ Channel membranes. B_{max} value of 0.5 pmol/mg and K_d value of 0.13 nM were obtained.

Figure 2B. In competition experiments, the radioligand was used at the K_d concentration. K_i values are the average of two independent experiments. A quantity of 2.4 µg of hERG K^+ Channel membranes was used per well. The assay was incubated for 1 h at room temperature. Signal was detected with a MicroBeta after filtration of the samples.

Ordering information

Membrane Target Systems

Product	Cat. No.	Size
hERG K^+ Channel	RBHERGM400UA RBHERGM000UA	400 assay units* Bulk

* *One assay unit is defined as the amount of membranes per well in a 96-well plate in a filtration assay.*

Complementary products

Radioligands

Product	Cat. No.	Size
BeKm-1, [^{125}I-Tyr^{11}]-	NEX412	10 and 25 µCi
Astemizole, [O-Methyl-^{3}H]-	NET1140	25 µCi, 250 µCi, 1 mCi

Note: [^{125}I-Tyr^{11}]-BeKm-1 is used for QC validation of the membrane product.

Microplates & Filtration

Product	Cat. No.	Size
OptiPlate™-96 White opaque 96-well microplate	6005299	200/box
OptiPlate™-384 White opaque 384-well microplate	6007299	200/box
IsoPlate-96 White 96-well Microplate with Clear Well	1450-515	100/box
UniFilter-96, GF/C	6005174	50/box
Filtermat A, GF/C	1450-421	100/PK
Filtermat A, 24-well	1450-422	100/PK
MeltiLex for MicroBeta® filters	140-441	100/PK

Assay Platforms

Product	Cat. No.	Size
FlashBlue™ GPCR	FBB001500MG FBB001002G	500 mg 2 g
Wheat Germ Agglutinin FlashPlate® PLUS, 96-well	SMP105A001PK	20/PK
Wheat Germ Agglutinin FlashPlate® HTS PLUS, 384-well	SMP411A001PK	10/PK
Image FlashPlate® WGA coated, 384-well	RMP111	40/PK

FlashBlue hERG K^+ Channel binding assay with [^{125}I]-BeKm-1

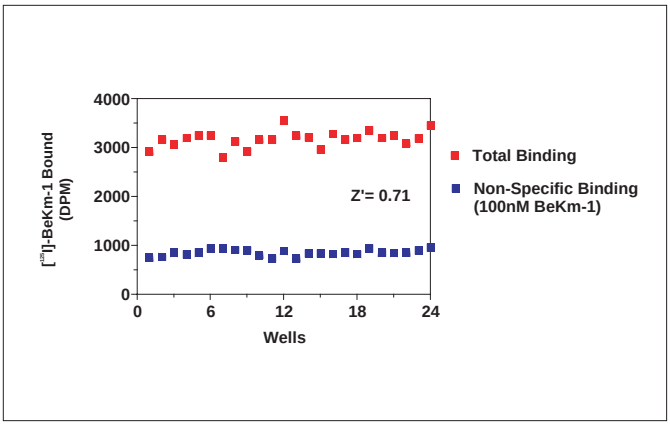


Figure 3. A homogeneous FlashBlue assay was developed with the radioligand [^{125}I]-BeKm-1. The Z' value was calculated according to the formula of Zhang *et al.*[5] A Z' value of 0.71 was obtained, demonstrating excellent performance. In this assay, a quantity of 5 µg of hERG K^+ Channel membranes and 125 µg FlashBlue GPCR beads were added per well. The radioligand was used at the K_d concentration. The assay was performed manually in a PerkinElmer® Isoplate™ 96-well in a total volume of 80 µL. The plate was incubated for 1 h at room temperature and spun for 5 min at low speed prior to reading with the MicroBeta.

Compatible Instrumentation

Product	Cat. No.	Size
Wallac MicroBeta® TriLux, 12 Detector, 32-shelf Model	1450-030	1/EA
TopCount® 12 detector, 96 and 384 format	C384V01	1/EA
ViewLux™ ultraHTS Microplate Imager	1430-0010	1/EA
UniFilter-96 Harvester	C961961	1/EA
UniFilter-96 Harvester (stainless steel)	C961962	1/EA
MicroBeta® Filtermate-96 Harvester	D961962	1/EA
MicroBeta® Filtermate-24 Harvester	D961241	1/EA

References

1. Chiu PJS *et al.* (2004) J. Pharmacol. Sci. **95**: 311-319.
2. Finlayson K *et al.* (2001) Eur. J. Pharmacol. **430:** 147-148.
3. Diaz GJ *et al.* (2004) J. Pharmacol. Toxicol. Methods. **50:** 187-99.
4. Angelo K *et al.* (2003) Pflugers Arch. **447**: 55-63.
5. Zhang JH *et al.* (1999) J. Biomol. Screen. **4**: 67-73.

For additional information, please do not hesitate to contact us

US & Canada toll free: 1-800-762-4000 prompt 3, 3, 1
European Headquarters PerkinElmer Life and Analytical Sciences: Tel: 800 906 642 or 39 039 2383-1
Austria Tel: (43) 0800111933 • **Belgium** Tel: (32) 0800-40-858 • **Denmark** Tel: (45) 80-88-42-36 • **Finland** Tel: (358) 080011718
France Tel: (33) 0805 111 333 • **Germany** Tel: (49) 08001810032 • **Italy** Tel: (39) 0800 906 642 • **Luxembourg** Tel: (352) 80026588
Netherlands Tel: (31) 0800 0234 490 • **Norway** Tel: (47) 800 18854 • **Spain** Tel: (34) 8000 99 164 • **Sweden** Tel: (46) 0200 887520
Switzerland Tel: (41) 0 800 000 015 • **United Kingdom** Tel: (44) 0800896046 • **Export** Tel: (39) 0392383275
e-mail at techsupport@perkinelmer.com or techsupport.europe@perkinelmer.com.

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer® precisely.

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

007468_01 Printed in USA