

# Cell-Based Potassium Ion Channel Screening with the FluxOR™ Assay

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## Abstract

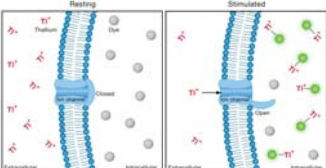
Invitrogen's Discovery Assays and Services (DAS) segment has recently developed and brought to market the FluxOR™ universal potassium ion channel assay kit. FluxOR™ is a cell-based assay tool to be used for High Throughput Screening (HTS) measurements of potassium channel activity. FluxOR™ is compatible with either wash or homogenous screening methods. With over 80 mammalian genes encoding functional and obligatory heteromeric protein subunits that must co-assemble, potassium ion channels are a broadly expressed target class with indications in a variety of disease and normal functions.

Using thallium influx as a surrogate indicator of potassium ion channel activity, the assay is based on the activation of FluxOR™, a novel fluorescent dye with a high affinity for thallium (Figure 1) that reports channel activity with a large fluorogenic response that is proportional to the number of open potassium channels on the cell.

In contrast to BTC-AM ester, (Weaver et al, Hoegaard et al), FluxOR™ dye is roughly tenfold more thallium sensitive, requiring much lower thallium concentrations in the medium for a larger signal window. This also means that the assay is carried out in a physiological, normal-chloride saline.

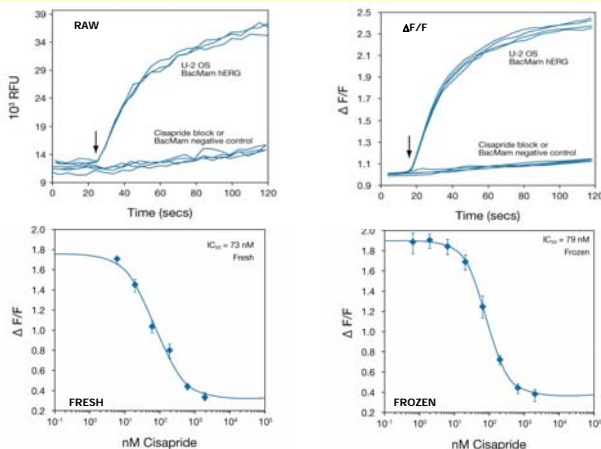
Here, we used BacMam gene delivery technology (Kost et al) to express different potassium ion channel targets. We then ran the FluxOR™ assay to identify and characterize clone-specific inhibitory compounds discovered within the ToxScreen™ Mini 1200 library.

## Figure 1 – FluxOR™ Assay



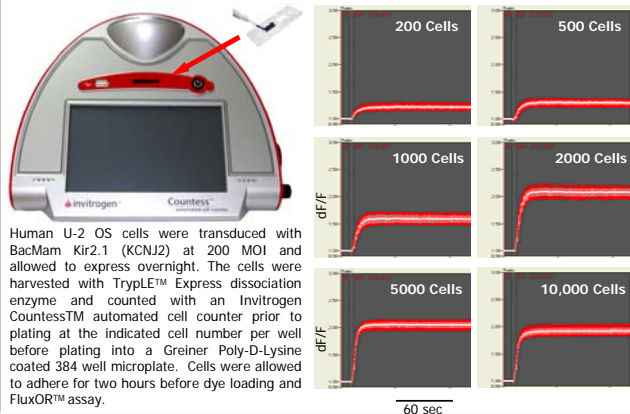
Cells are loaded with FluxOR™, a thallium sensitive dye. During the assay, a small amount of thallium is added to the outside of the cells with a stimulus. Thallium flows into the cells in direct proportion to the number of open potassium channels, resulting in the evolution of a fluorescent signal. The excitation/emission of FluxOR™ is optimal with standard FITC (488nm ex 520 - 540nm em) filter settings on any HTS platform.

## Figure 2 – Fresh or Frozen Cell Capabilities using the FluxOR™ kit



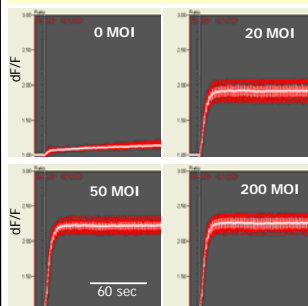
U-2 OS cells transduced with BacMam hERG and measured on a Molecular Devices FlexStation™ plate reader. The y-axis shows raw (RFU) and normalized (dF/F) fluorescence after potassium stimulation (arrow) at 20 seconds shown on the x-axis. Bottom panels show the results of freshly transduced cells and cells which had been transduced and then frozen in liquid N<sub>2</sub>.

## Figure 3 – Optimizing Cell Density in the FluxOR™ assay with the Countess™ Automated Cell Counter



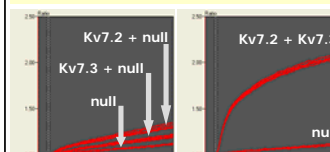
Human U-2 OS cells were transduced with BacMam Kir2.1 (KCNJ2) at 200 MOI and allowed to express overnight. The cells were harvested with TrypLE™ Express dissociation enzyme and counted with an Invitrogen Countess™ automated cell counter prior to plating at the indicated cell number per well before plating into a Greiner Poly-D-Lysine coated 384 well microplate. Cells were allowed to adhere for two hours before dye loading and FluxOR™ assay.

## Figure 4 – MOI Study with BacMam Kir2.1



U2OS cells (ATCC #HTB-96) were transduced with BacMam Kir2.1 (KCNJ2), delivered at the indicated MOI. The cells were harvested for resuspension in divalent free PBS at 1 x 10<sup>6</sup> cells/mL. Aliquots were prepared in 15 mL tubes with 1 mL of cells (10<sup>6</sup>) viral stock needed to achieve the indicated (MOI). This suspension was incubated at room temperature for 60 minutes before adding complete medium and plating at 5,000 cells per well. The FluxOR™ assay was run on them the following day.

## Figure 5 – Co-Expression of BacMam Kv7.2 and Kv 7.3

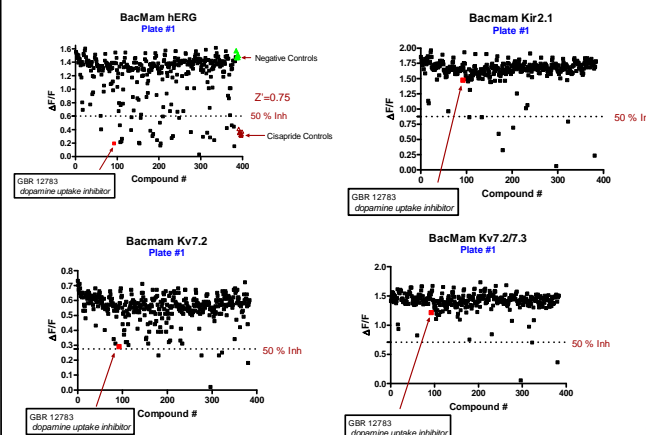


U2OS cells were transduced with BacMam null, Kv7.2 plus null, Kv7.3 plus null, or Kv7.2 + Kv7.3 at 200 MOI and tested in the FluxOR™ assay the following day. Kv7.2 and 7.3 (KCNO2 and KCNO3) co-assemble to underlie the M current in CNS.

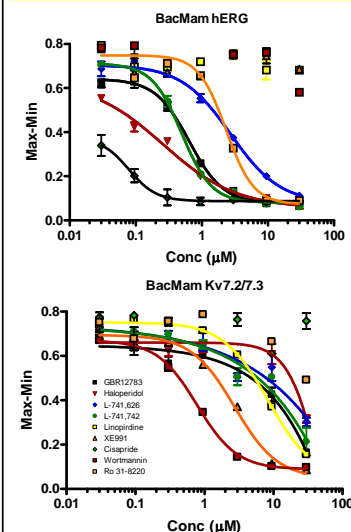
## Toxris Plate 1



## Figure 6 – FluxOR™ Screening Results with Hamamatsu FDSS



## Figure 7 – Cherry Picked Compounds



## Figure 8 – IC50 Values

Compound	hERG IC <sub>50</sub>	Kv7.2/7.3 IC <sub>50</sub>	Description	Reference
GBR 12783	645 nM	>10 μM	α-1 adrenergic receptor antagonist	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
Haloperidol	210 nM	>10 μM	antipsychotic	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
L-741,826	2.73 μM	>10 μM	anticholinergic	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
L-741,742	695 nM	>10 μM	anticholinergic	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
Linopirdine	>10 μM	779 μM	potassium channel activator	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
AE991	>10 μM	84 μM	potassium channel activator	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
Wortmannin	>10 μM	2.7 μM	phosphatidylinositol 3-kinase inhibitor	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
Ro 31-8220	2.3 μM	>10 μM	potassium channel activator	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)
Cisapride	78 nM	>10 μM	5-HT <sub>4</sub> receptor agonist	Journal of Pharmacology and Experimental Therapeutics 277:101-107 (1996)

## References

Weaver et al Journal of Biomolecular Screening 9 (8) 671-7  
 Hoegaard et al British Journal of Pharmacology (151) 655-665  
 Kost TA et al Nature Biotechnology (23) 567-575  
 Kost TA et al Drug Discovery Today 12(9-10):396-403