

# FluxOR™, a Universal Potassium Channel Assay

## Screening BacMam Delivered and Stably Expressed Targets

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Photonics

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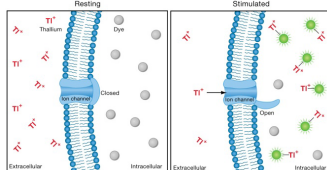
### Abstract

Potassium ion channels are important targets in drug discovery and safety testing for which current assays suffer expense, low throughput or lack of proximity to the target. A robust and homogenous assay that allows for functional HTS measurements of potassium channel activity would give the screening community a useful tool for the interrogation of potassium channels in compound library screening.

Invitrogen's Molecular Probes Labeling and Detection Technologies has developed an equilibrium based assay for HTS measurement of potassium ion channel activity called the FluxOR™ Thallium Assay Kit. 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 competing thallium flux methods or published applications with BTC, (Weaver et al, Hoegaard et al), FluxOR™ dye requires lower thallium concentrations for a larger signal window and normal chloride conditions.

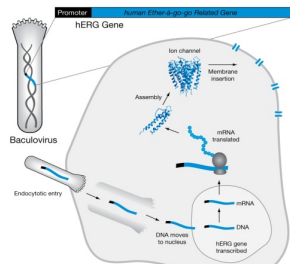
Combined with BacMam Target delivery (Kost et al, Ames et al), many potassium channels, including hERG, are now amenable to high throughput screening in a variety of cellular backgrounds, including primary human aortic smooth muscle, allowing flexibility of genetic context for maximum biological relevance.

### Figure 1 – FluxOR™ Assay



Cells are loaded with FluxOR™, a thallium sensitive dye. During the assay, a small amount of thallium (2 mM final) is added to the outside of the cells with a stimulus, e.g. 10 mM potassium for voltage gated channels or calcium ionophore for calcium activated potassium channels. 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 (488/525 nm) filter settings on the Hamamatsu FDSS or other HTS platform.

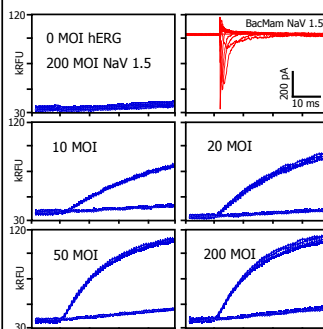
### Figure 2 – BacMam Target Delivery



BacMam delivery of potassium ion channels combined with the FluxOR™ assay tool provides an easy-to-use equilibrium based measurement of potassium channel activity for interrogation of a multiple ion channels in a variety of cellular and genetic contexts.

Cells: U-2 OS >90%  
COS-7 >90%  
HEK293 80-90%  
CHO 50-80%  
HeLa 60-70%  
Primary Human Aortic Smooth Muscle >70%

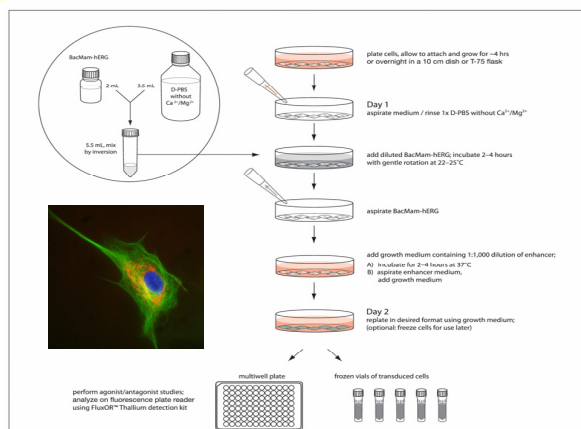
### Figure 3 – Titratable Expression



U-2 OS cells transduced with BacMam-hERG, activity measured with FluxOR™. Panel 2 shows patch clamp records from HEK 293 cells expressing BacMam Nav1.5 (Heka Electronic).

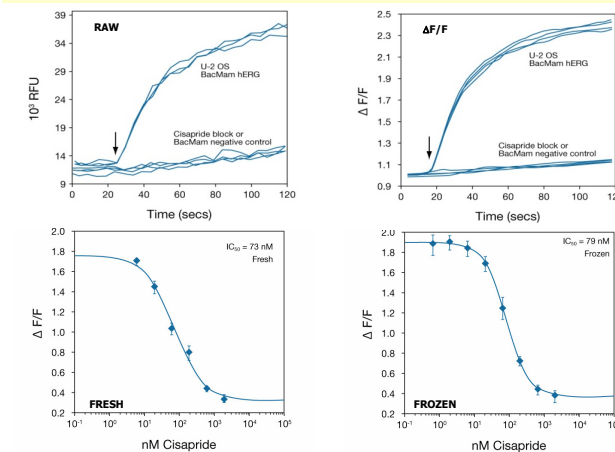
Channels: Kv11.1 (hERG, KCNH2)  
Kir2.1 (KCNJ2)  
Kv7.2 (KCNQ2)  
Kv7.3 (KCNQ3)  
Nav1.5 (SCN5A)  
Titration of multiple subunits

### Figure 4 – BacMam Target Delivery Workflow – Fresh or Frozen?



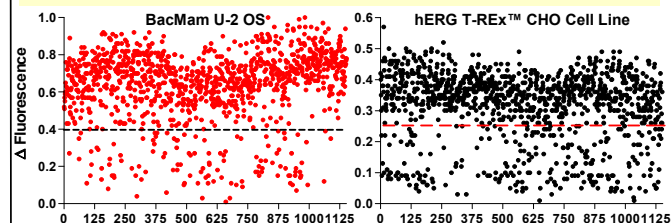
### Figure 5 – BacMam hERG K+ Channel Delivery with FluxOR™ Assay

#### Fresh or Frozen Cell Capabilities



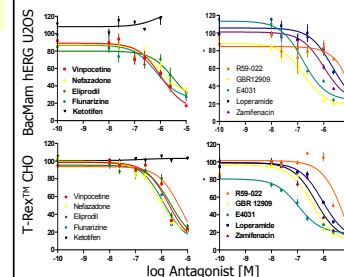
U-2 OS cells transduced with BacMam hERG and measured on a Molecular Devices FlexStation™ plate reader. The y-axis shows raw (RFU) and normalized (ΔF/F) fluorescence after potassium stimulation (arrow) at 20 seconds shown on the x-axis. Bottom panels show the results of freshly transduced cells and cells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

### Figure 6 – FluxOR™ Screening Results with Hamamatsu FDSS on hERG in BacMam Transduced U-2 OS and T-Rex™ CHO Cells



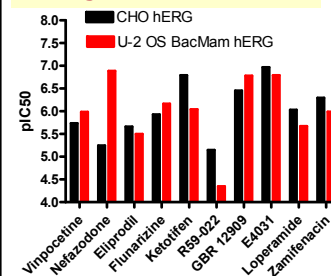
U-2 OS cells transduced with BacMam hERG and hERG expressing T-Rex™ CHO cells interrogated for activity in the FluxOR™ assay against the Tocriscreen™ miniscreen compound library. U2OS cells (panel 1) were transduced with BacMam hERG at an MOI of 200, allowed to grow overnight and plated at 5,000 cells per well in 384 well Greiner PDL microplates four hours prior to FluxOR™ screening. hERG T-Rex™ CHO cells (panel 2) were split into complete medium plus 1 μg/mL doxycycline in 384 microwell plates 24 hours before screening at 5,000 cells per well. The compound library was prepared at 100 μM in FluxOR™ assay buffer + 10 % DMSO and 2 μL was added to 18 μL of FluxOR dye-loaded cells for a final concentration of 10 μM and allowed to equilibrate for 30 minutes before running the assay. Final thallium in the assay was 2 mM and potassium was 10 mM after 1:5 dilution (5 μL added to 20 μL). Final DMSO in the assay was 1%.

### Figure 7 – Selected Hits



Dose response curves of selected responders (and one negative) picked out from the Tocriscreen™ miniscreen compound library. U-2 OS and CHO results reflected as percent deviation from change in fluorescence as defined by 60 seconds following stimulation.

### Figure 8 – IC50 Values



### Conclusions

FluxOR™ thallium detection provides a versatile, all inclusive solution to potassium channel drug discovery and screening. Kits are all inclusive of the reagents for assay development and full scale screen. Similar results were obtained in parallel screens with BacMam Kir2.1 as well as hERG T-Rex™ 293 cells, with Z' values better than 0.7.

### 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