molecular probes
life technologies

FluxOR™ Potassium Ion Channel Assay

Catalog nos. F10016, F10017

Table 1 Contents and storage

Material	Amount		Concentration	Storage*	Stability
	F10016	F10017			
FluxOR™ Reagent (Component A)	1 vial	10 vials	1000X after reconstitution in DMSO	• ≤–20°C • Desiccate • Protect from light	When stored as directed, the product is stable for at least 6 months.
FluxOR™ Assay Buffer (Component B)	25 mL	250 mL	10X Concentrate	2–8°C	
PowerLoad™ Concentrate (Component C)	1 mL	10 mL	100X Concentrate		
Probenecid (Component D)	2 × 77 mg	20 × 77 mg	100X after solubilization in water	• ≤–20°C • Desiccate	
FluxOR™ Chloride-free Buffer (Component E)	25 mL	250 mL	5X Concentrate	2–8°C	
Potassium sulfate (K_2SO_4) Concentrate (Component F)	20 mL	200 mL	125 mM in water		
Thallium sulfate (Tl_2SO_4) Concentrate (Component G)	20 mL	200 mL	50 mM in water		
Dimethyl sulfoxide (DMSO) (Component H)	100 µL	1 mL	100%	• ≤25°C • Desiccate	

* All products are shipped on dry ice and can be stored at –20°C.

Number of assays: Sufficient reagents are supplied for 10 or 100 microplates (96-or 384-well), based on the protocol below. Each vial of Component A is sufficient for assaying 10 microplates.

Approximate fluorescence excitation/emission maxima: 490/525 nm.

Introduction

The FluxOR™ Potassium Ion Channel Assay is a solution for high throughput screening (HTS) of potassium ion channel and transporter activities. The FluxOR™ assay takes advantage of the well described permeability of potassium channels to thallium (Tl^+) ions.[1,2] When thallium is added to the extracellular solution with a stimulus to open channels, thallium flows down its concentration gradient into the cells, and channel or transporter activity is detected with a proprietary indicator dye that increases in cytosolic fluorescence. In this way, the fluorescence reported in the FluxOR™ system becomes an indicator of any ion channel activity or transport process that allows thallium into cells.

For Research Use Only. Not for use in diagnostic procedures.

The FluxOR™ Potassium Ion Channel Assay provides all necessary buffers and solutions needed for 10 (Cat. no. F10016) or 100 (Cat. no. F10017) 96- or 384-well microplates. The kits allow maximum flexibility and ease of operation in a homogenous format against a variety of potassium ion channel and transporter targets. The FluxOR™ Potassium Ion Channel Assay has been demonstrated for use with CHO and HEK293 cells stably expressing hERG, as well as U-2 OS cells transiently transduced with BacMam-hERG or other potassium ion channels that are available in our suite of BacMam potassium channel target reagents. The assay does not utilize quencher dyes, nor does it have an absolute requirement for chloride-free buffer in the loading and detection steps of the protocol.

Assay principle

The FluxOR™ reagent (Component A) is a fluorogenic indicator dye, which is loaded into cells as a membrane-permeable AM ester (Figure 1, page 3). The FluxOR™ reagent is dissolved in DMSO (Component H) and further diluted with FluxOR™ assay buffer (Component B), a physiological HBSS (Hank's balanced salt solution), for loading into cells. Loading is assisted by the proprietary PowerLoad™ concentrate (Component C), a formulation of Pluronic® surfactants, which acts to disperse and stabilize AM ester dyes for optimal loading in aqueous solution.

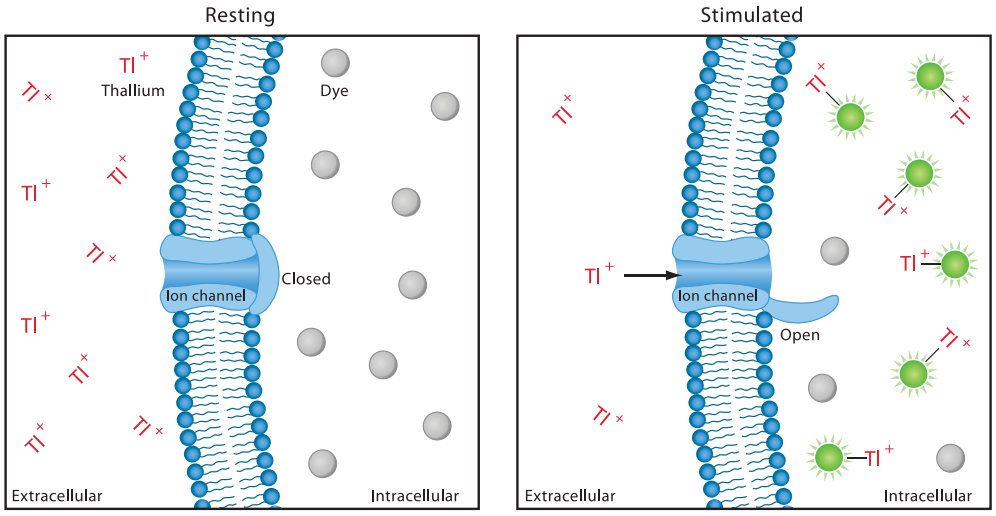
Once inside the cell, the non-fluorescent AM ester form of the FluxOR™ dye is cleaved by endogenous esterases into a fluorogenic thallium-sensitive indicator. The thallium-sensitive form is retained in the cytosol and its extrusion is inhibited by water-soluble Probenecid (Component D), which blocks organic anion pumps. For most applications, cells are loaded with the dye at room temperature. For best results, the dye-loading buffer is then replaced with fresh, dye-free assay buffer, composed of physiological HBSS containing Probenecid, before the HTS assay.

During the HTS assay, a small amount of thallium is added to the cells with a stimulus solution that opens potassium-permeant ion channels with a mild depolarization or agonist addition. Thallium then passes into cells through open potassium channels according to a strong inward driving force. Upon binding cytosolic thallium, the de-esterified FluxOR™ dye exhibits a strong increase in fluorescence intensity at its peak emission of 525 nm. Baseline and stimulated fluorescence is monitored in real time to give a dynamic, functional readout of thallium redistribution across the membrane with no interference from quencher dyes. The FluxOR™ assay may also be used to study enzymatic potassium transport processes that accommodate the transport of thallium into cells.[2] Voltage gated potassium channels such as hERG (Cat. no. B10019), Kv1.3 (Cat. no. B10332), Kv2.1 (Cat. no. B10333), and Kv7.2/7.3 (Cat. no. B10147) are opened by the co-administration of potassium and thallium in the stimulus buffer. Resting and inward rectifier potassium channels such as Kir1.1 (Cat. no. B10334) and Kir2.1 (Cat. no. B10146) are assayed by adding stimulus buffer with thallium alone, as are potassium ion transporters and cells interrogated for GPCR activity by way of coupling to calcium (KCa) or G protein activated (GIRK) potassium channels.

The unique FluxOR™ reagent formulation allows the use of the dye in physiological saline, without the need to load or assay cells in chloride-free conditions. This is a major advantage over traditional approaches to thallium flux assays that utilize completely chloride-free conditions to load cells with the dye.[3] Thallium chloride is an insoluble precipitate that forms when concentrations of free thallium and chloride in the solution are greater than about 4 mM. Because the FluxOR™ reagent is extremely sensitive to thallium, the reagent is added to cells for most applications at a final concentration of 2 mM after dilution into the assay plate. The kits include concentrates of Tl_2SO_4 and K_2SO_4 that allow you to optimize the dose of surrogate (thallium) and stimulus (potassium) used together in the assay to depolarize voltage gated potassium channels.

For ligand gated channels, resting potassium channels, and potassium transporters, the extra potassium is left out of the stimulus buffer, and the stimulus buffer is composed of the channel opener and thallium concentrate, which are necessarily prepared in the chloride-free buffer provided in the kit. However, for low expressing or poorly thallium-permeant targets requiring high extracellular thallium concentrations, enough chloride-free buffer is supplied for the assay and stimulation, allowing for maximum ease and flexibility of use against a wide range of targets. In these cases, cells are still loaded with the FluxOR™ reagent in FluxOR™ assay buffer, a normal HBSS solution, which is removed and replaced with 1X chloride-free buffer containing Probenecid prior to running the assay.

Figure 1. Thallium redistribution in the FluxOR™ assay. Basal fluorescence from cells loaded with FluxOR™ dye is low, as shown in the left panel, until potassium channels are stimulated. When thallium is added to the assay with the stimulus, the thallium flows down its concentration gradient into the cells, activating the dye as shown in the right panel.



Before Starting

Materials required but not provided

- Cells expressing potassium channel(s) of interest and corresponding culture medium for the growth and maintenance of the cells
- Vessels for preparing the assay, loading, and stimulus buffers
- 96- or 384-well microplates

Caution

Thallium sulfate (Component G) is toxic. Use caution when handling thallium sulfate and all solutions prepared containing thallium sulfate. Properly dispose of any waste containing thallium in compliance with all pertaining local regulations.

DMSO (Component H) is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations.

Thawing and preparing reagents

1.1 To prepare 100X Probenecid stock solution, add 1 mL of deionized water to each vial containing 77 mg of Probenecid (Component D). Vortex the vial until the powder is completely dissolved. Use the solution on the day of preparation or aliquot and store any unused portions at –20°C for up to 6 months. Avoid repeated freeze-thaw cycles.

Note: 200 µL of 100X Probenecid is needed per microplate.

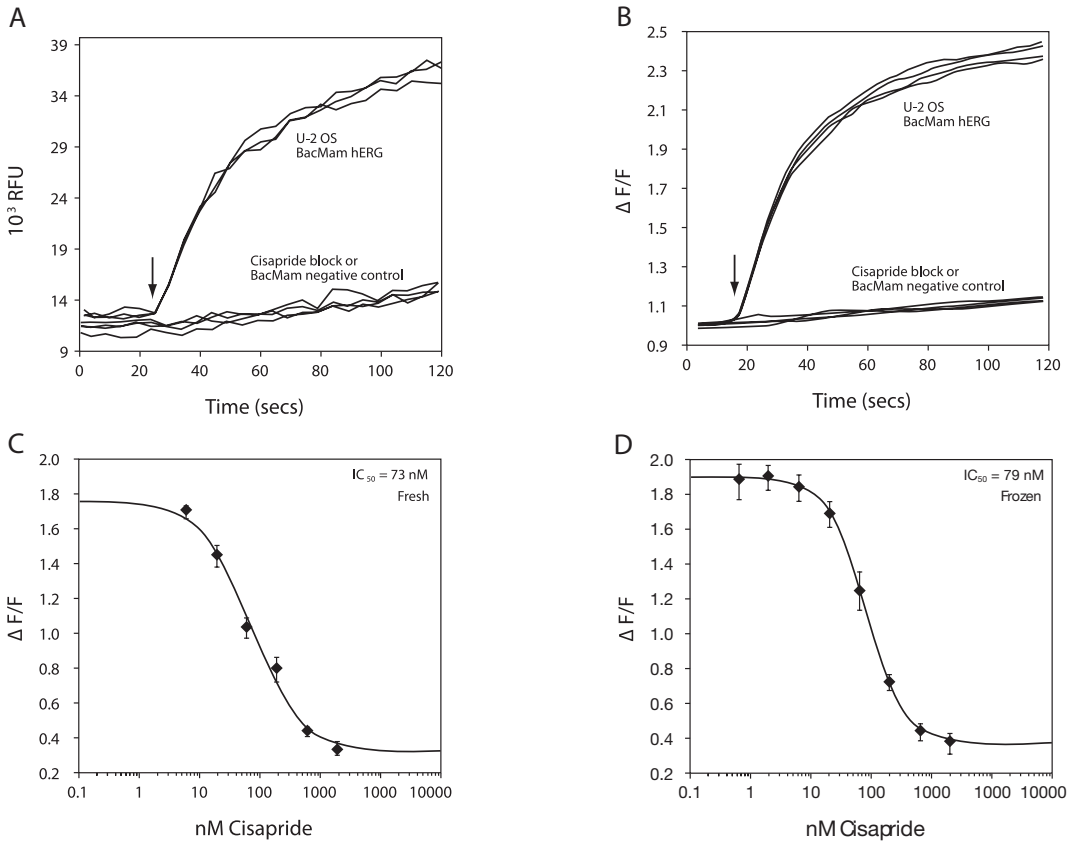
1.2 To prepare 1000X dye stock solution, thaw DMSO (Component H) and briefly centrifuge the vial. Add 100 µL DMSO to a single vial of FluxOR™ reagent (Component A) to yield a 1000X dye stock solution. Vortex briefly to dissolve the dye completely. The dye stock solution should be colorless. Aliquot and store any unused portions at –20°C, protected from light and moisture. Avoid repeated freeze-thaw cycles.

Note: 10 µL of 1000X dye stock solution is needed per microplate.

1.3 Thaw Components B, C, E, F, and G and store at 4°C until use.

Note: To dissolve any precipitate that might form due to freezing of Powerload™ concentrate (Component C), warm Component C to 37°C.

Figure 2. U-2 OS cells were transduced with BacMam-hERG as described in the BacMam hERG protocol (MP 10019). Freshly transduced cells were assayed with the FluxOR™ Thallium detection kit or the cells were stored frozen, and then thawed on the day of the assay. The thallium flux assays were performed on a FlexStation® II 384 (Molecular Devices, Sunnyvale, CA). **Panel A** shows raw data obtained in a FluxOR™ assay determination of hERG activity in U-2 OS cells kept frozen until the day of use. The arrow indicates the addition of the thallium/potassium stimulus. Upper and lower traces show data taken from the minimum and maximum doses, respectively, of cisapride (inhibitor of the hERG channel) used in the determination of the dose-response curves. Raw pre-stimulus peak and baseline values were boxcar averaged and normalized to generate the data shown in **panel B**, which demonstrate the time-dependent fold increase in fluorescence over baseline (ΔF/F). **Panel C** shows data generated in a dose-response determination of cisapride block on BacMam hERG expressed in fresh U-2 OS cells 24 hour after transduction. **Panel D** shows data obtained from U-2 OS cells transduced with BacMam-hERG, frozen after a 24 hour incubation and stored for 2 weeks in liquid nitrogen. Cells were thawed and plated 4 hours prior to the assay. Error bars indicate standard deviation, n=4 per determination.



The Quick Start Protocol is designed as a starting point for interrogating most potassium channels. Refer to the Experimental Protocol for suggested modifications to buffer composition and additional detailed steps.

2.1 For each microplate prepare 10 mL **loading buffer.** Add the components in the order indicated:

PowerLoad™ concentrate, 100X (Component C)	100 µL
FluxOR™ reagent, reconstituted in DMSO (Step 1.2)	10 µL
Deionized water	8.8 mL
FluxOR™ assay buffer, 10X (Component B)	1 mL
Probenecid, reconstituted in deionized water (Step 1.1)	100 µL
Total Volume	10 mL

2.2 Remove media from cells and add 20 µL (for a 384-well plate) or 80 µL (for a 96-well plate) of loading buffer (Step 2.1) to each well.

2.3 Protect samples from light and incubate at 18–24°C for 60 minutes. During this time, prepare the assay buffer and the stimulus buffer.

2.4 Prepare 10 mL of **assay buffer:**

Deionized water	8.7 mL
FluxOR™ assay buffer, 10X (Component B)	1 mL
1 M HEPES	200 µL
Probenecid, reconstituted in deionized water (Step 1.1)	100 µL
Total Volume	10 mL

IMPORTANT! Adjust pH to 7.4 with NaOH.

2.5 Prepare 10 mL of **stimulus buffer** (+K for voltage gated K channels):

	$+K^+$	$-K^+$
Deionized water	2.5 mL	3.5 mL
FluxOR™ chloride-free buffer, 5X (Component E)	1 mL	1 mL
K_2SO_4 concentrate (Component F)	1 mL	–
Tl_2SO_4 concentrate (Component G)	0.5 mL	0.5 mL
Total Volume	5 mL	5 mL

2.6 Remove loading buffer and replace with 20 µL (for a 384-well plate) or 100 µL (for a 96-well plate) of assay buffer to each well.

2.7 *Optional*: Add test compounds and incubate for 10–30 minutes at 18–24°C.

2.8 Perform assay using a kinetic dispense microplate reader. Set up the instrument with standard FITC green filters or set the excitation/emission wavelengths to 460–490 nm excitation and the emission wavelengths to 520–540 nm. Add stimulus buffer after 10 seconds of recording (5 µL for a 384-well plate or 20 µL for a 96-well plate). Read plate every 1–2 seconds for 1–3 minutes.

The following protocol describes an initial starting point for using the FluxOR™ Potassium Ion Channel Assay to interrogate potassium ion channel activity. Further signal optimization may be necessary, which you can easily achieve by adjusting the levels of potassium and thallium in the Stimulus Buffer.

Important Load the cells within 20 minutes of preparing the loading buffer (Step 3.2). Discard any excess loading buffer.

HTS assay for one microplate of cells

3.1 Plate cells expressing potassium ion channels of interest onto Poly-D-Lysine coated microplates. Allow the cells to recover and adhere for at least 4 hours.

Note: The use of Poly-D-Lysine coated plates helps cells to attach and stay in place during the kinetic read; however, these special plates are not a requirement for assay performance.

3.2 To prepare **loading buffer**, thaw a vial containing 1000X dye stock solution in DMSO (prepared in Step 1.2) to room temperature and mix the following reagents in a 15 mL conical tube. Add the reagents in the order described below to maximize the dye solubility.

PowerLoad™ concentrate, 100X (Component C)	100 µL
FluxOR™ reagent, reconstituted in DMSO (Step 1.2)	10 µL

Mix well for 10 seconds, then add:

Deionized water	8.8 mL
FluxOR™ assay buffer, 10X (Component B)	1 mL
Probenecid, reconstituted in deionized water (Step 1.1)	100 µL

Mix well to ensure complete dissolution of the components and transfer the loading buffer to a trough for dispensing into the assay plate.

Note: For probenecid-sensitive applications, or applications where Pluronic® surfactants are not desired, perform the assay without these reagents. However, significant loss of signal may be observed in their absence. This varies strongly with cell type and should be tested for each new target and cell line being used.

3.3 To load cells, completely aspirate the medium and replace with 80 µL loading buffer per well for a 96-well plate or 20 µL per well for a 384-well plate. Incubate at room temperature for 45–90 minutes, **protected from light**. During this time, prepare the **assay buffer** and the **stimulus buffer**.

Note: Optimal loading times and incubation temperatures vary for different cell types. Incubate poorly loading cells at 37°C to facilitate dye entry.

3.4 To prepare **assay buffer**, add 1 mL of FluxOR™ assay buffer (Component B), 100 µL of water soluble, 100X Probenecid (Component D), and 200 µL of 1 M HEPES to 8.7 mL deionized water for each microplate. Mix well, adjust the pH to 7.4 with NaOH, and transfer to a trough for dispensing into the assay plate.

3.5 To prepare **stimulus buffer**, combine the following reagents and mix well. When used as described in the protocol, the stimulus buffer dilutes 1:5 into the assay buffer during the experiment, resulting in final concentrations of 10 mM free K^+ and 2.0 mM free Tl^+. For ligand gated ion channels or resting inward rectifier channels, omit K_2SO_4 (Component F) and use water instead. Prepare ligands as needed into the stimulus buffer.

	$+K^+$	$-K^+$
Deionized water	2.5 mL	3.5 mL
FluxOR™ chloride-free buffer, 5X (Component E)	1 mL	1 mL
K_2SO_4 concentrate (Component F)	1 mL	–
Tl_2SO_4 concentrate (Component G)	0.5 mL	0.5 mL

Note: For voltage gated potassium channels, varying the amount of K_2SO_4 in the stimulus buffer will help determine the optimal dosage for opening a particular potassium channel. Since potassium at high levels competes with thallium for entry, choose a K_2SO_4 concentration that produces the widest signal window. 10 mM added potassium (5 mM K_2SO_4) is optimal for most voltage gated channels.

3.6 After 45–90 minutes of incubation, remove the loading buffer and replace with 80 µL of assay buffer (prepared in Step 4.4) per well for a 96-well plate or 20 µL per well for a 384-well plate.

Note: You should perform the assay as soon as possible after exchanging the loading buffer with fresh assay buffer.

3.7 *Optional*: Add test compounds and incubate for 10–30 minutes. You may pre-incubate the test compounds with the cells.

3.8 To perform the assay, set up the plate reader as needed with with an FITC green filter set or set the excitation/emission wavelengths to 460–490 nm excitation and 520–540 nm emission. Read the assay plate every 1–2 seconds, obtaining 10–30 seconds of baseline before adding 20 µL per well of stimulus buffer for 96-well plates or 5 µL per well of stimulus buffer for 384-well plates.

Continue reading the plate for 60–180 seconds after adding the stimulus to determine where the optimal signal is observed above background, using the complete drug block or negative expression control as a reference. In most cases, the strongest signal above background is observed at 60–90 seconds following the addition of the stimulus.

Using high thallium concentrations to detect difficult targets

When using high thallium concentrations to detect difficult targets, use FluxOR™ chloride-free buffer (Component E) as the assay buffer in Step 4.4 after the cell loading procedure described above.

To prepare the working assay buffer, mix 2 mL of FluxOR™ chloride-free buffer (Component E) with 8 mL of deionized water and 100 µL Probenecid. These conditions may help in obtaining signals from poorly expressing targets or targets with limited thallium permeability. Sufficient FluxOR™ chloride-free buffer (Component E) and thallium concentrate (Component G) are included in the kit to run the assay in conditions, allowing up to 8 mM free thallium after dilution into the microtiter plate.

References

1. Assay Drug Dev Technol 6, 765 (2008); **2.** J Biol Chem 284, 14020 (2009); **3.** J of Biomol Screen 9, 671 (2004); **4.** J Gen Physiol 61, 669 (1973); **5.** Nature Biotech 23, 567 (2005); **6.** Trends in Biotech 20, 173 (2002); **7.** Drug Discovery Today 12, 396 (2007); **8.** Assay Drug Dev Technol 5, 417 (2007); **9.** Expert Opin Drug Discov 2, 1669 (2007); **10.** Adv Virus Res 68, 255 (2006); **11.** Expression and Analysis of Recombinant Ion Channels: From Structural Studies to Pharmacological Screening, Clare, J.J. & Trezise, D.J. Eds. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp.79-109.

Frequently Asked Questions

Q: Why is there no change in signal from my cells?
A: Make sure there is thallium present in the stimulus buffer only.

Q: How can I increase the signal window?
A: Prepare fresh stimulus buffer, and run a matrix of thallium and potassium doses to determine the optimal ratio for your target of interest.

Q: What causes a small signal window?
A: There are several possible reasons for a small signal window: 1) low level expression of target, 2) low thallium permeability of target, 3) cells were loaded too long (>2 hours), 4) cells were loaded too short (<30 minutes), or 5) Probenecid was not included.

Q: Why is there no detectable signal from the cells?
A: Cells may not have been loaded with dye. FluxOR™ dye should be readily visible with an FITC filter on a fluorescence microscope. Check for even, cytoplasmic labeling of the cells after loading. If cells are not labeled, check that the dye was properly resuspended and stored, and that the reagents in the loading buffer were mixed in the proper order.

Q: Why I am getting high variability from well to well?
A: Removal of solution from the wells is the likely cause. Make sure to aspirate or remove all culture medium before loading the cells with the loading buffer. Also, carefully remove all of the dye before replacing it with fresh assay buffer. Ensure that cells are not removed during the process. An additional wash step following the removal of the dye may also increase fidelity in the assay.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
F10016	FluxOR™ Potassium Ion Channel Assay *for 10 microplates*	1 kit
F10017	FluxOR™ Potassium Ion Channel Assay *for 100 microplates*	1 kit
Related Products		
B10019	BacMam hERG *for 10 microplates*	1 kit
B10332	BacMam Kv1.3 *for 10 microplates*	1 kit
B10333	BacMam Kv2.1 *for 10 microplates*	1 kit
B10334	BacMam Kir1.1 *for 10 microplates*	1 kit
P10020	PowerLoad™ concentrate, 100X	5 mL
P36400	Probenecid, water soluble	10 × 77 mg

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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