

FluxOR™ Red Potassium Ion Channel Assay

Catalog Number F20018, F20019

Pub. No. MAN0016213

Rev. A.0

Table 1 Contents and storage

Material	Amount		Concentration	Storage*
	F20018	F20019		
FluxOR™ Red Reagent (Component A)	2 vials** (for 2 plates)	10 vials** (for 10 plates)	1000X after reconstitution in DMSO	<ul style="list-style-type: none">• -25°C to 8°C• Desiccate• Protect from light
Dimethyl sulfoxide (DMSO) (Component B)	100 µL	200 µL	N/A	<ul style="list-style-type: none">• -25°C to 30°C• Desiccate
FluxOR™ Assay Buffer (Component C)	4 mL	20 mL	10X Concentrate	-25°C to 8°C
PowerLoad™ Concentrate (Component D)	400 µL	2 × 1 mL	100X Concentrate	
Probenecid (Component E)	1 × 77 mg	2 × 77 mg	100X after solubilization in water	<ul style="list-style-type: none">• -25°C to 30°C• Desiccate
Chloride-free Stimulus Buffer (Component F)	4 mL	20 mL	5X Concentrate	-25°C to 8°C
Potassium Sulfate (K ₂ SO ₄) Concentrate (Component G)	4 mL	20 mL	125 mM in water	
Thallium Sulfate (Tl ₂ SO ₄) Concentrate (Component H)	4 mL	20 mL	50 mM in water	
FluxOR™ Background Suppressor (Component I)	4 mL	20 mL	10X Concentrate	
<p>* All products are shipped on wet ice. When stored as directed, the product is stable for at least 6 months upon receipt.</p> <p>** Each vial contains sufficient reagents for assaying 1 microplate (96-or 384-well).</p> <p>N/A: Not applicable.</p>				
<p>Number of assays: Sufficient reagents are supplied for 2 (Cat. No. F20018) or 10 (Cat. No. F20019) microplates (96-or 384-well), based on the protocol below.</p>				
<p>Approximate fluorescence excitation/emission maxima: 560/590 nm.</p>				

Product information

The FluxOR™ Red Potassium Ion Channel Assay is a solution for high-throughput screening (HTS) of potassium ion channel and transporter activities. The FluxOR™ Red assay takes advantage of the 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 the FluxOR™ Red indicator dye that increases in cytosolic fluorescence. In this way, the fluorescence reported in the FluxOR™ Red system becomes an indicator of any ion channel activity or transport process that allows thallium into cells.

The FluxOR™ Red Potassium Ion Channel Assay provides all necessary buffers and solutions needed for performing the assay in 2 (Cat. No. F20018) or 10 (Cat. No. F20019) 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.

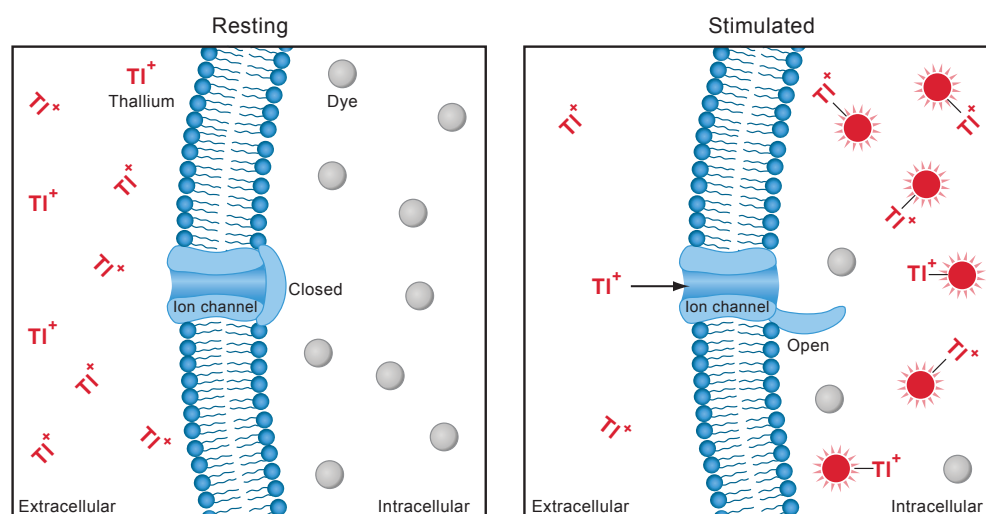


Figure 1. Thallium redistribution in the FluxOR™ Red assay. Basal fluorescence from cells loaded with FluxOR™ Red dye is low prior to stimulation of the potassium channels (left panel). When thallium is added to the assay with the stimulus, the thallium flows down its concentration gradient into the cells, activating the FluxOR™ Red dye (right panel).

Materials required but not provided

- Cells expressing potassium channels 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 H) 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 B) 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.

Before you begin

Thaw and prepare reagents

- 1.1 To prepare 100X Probenecid stock solution, add 1 mL of deionized water to each vial containing 77 mg of Probenecid (Component E) and vortex 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 FluxOR™ Red dye stock solution, thaw the DMSO (Component B) and briefly centrifuge the vial. Add 10 μL of DMSO to a single vial of FluxOR™ Red Reagent (Component A) to yield enough dye for one microplate. Vortex briefly to dissolve the dye completely and centrifuge to recover the full amount needed per microplate. 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 prepared from 1 vial of Component A is needed per microplate.

- 1.3 Thaw Components C, F, G, and H at room temperature, and store at 4°C until use.
- 1.4 Thaw the PowerLoad™ Concentrate (Component D) and the FluxOR™ Background Suppressor (Component I) at 37°C to ensure complete dissolution of the concentrated stock and to remove any precipitate that might have formed during storage. Store at 4°C until use.
- 1.5 Mix all previously frozen solutions well before use.

Prepare cells Resuspend the cells expressing the potassium ion channels of interest to 2×10^5 cells/mL in complete growth medium, and plate onto Poly-D-Lysine-coated microplates (80 μL /well for 96-well plates or 20 μL /well for 384-well plates). Allow the cells to recover and adhere for at least 4 hours.

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

Note: Cell culture conditions and plating density should be optimized for each target and cell type.

Methods

In the standard FluxOR™ Red Potassium Ion Channel Assay protocol, the cell culture medium is removed and the cells are loaded for 60 minutes with 1X Loading Buffer. The Loading Buffer is then removed and replaced with Dye-free Assay Buffer and the FluxOR™ Background Suppressor. Cells are then treated with drug or control, and the intracellular fluorescence is measured in a kinetic assay.

Prepare 1X Loading Buffer

2.1 For each microplate prepare 10 mL of 1X Loading Buffer. Add the components in the order indicated:

PowerLoad™ Concentrate, 100X (Component D)	100 µL
FluxOR™ Red Reagent, reconstituted in DMSO (Step 1.2)	10 µL
Deionized water	8.8 mL
FluxOR™ Assay Buffer, 10X (Component C)	1 mL
Probenecid, reconstituted in deionized water (Step 1.1)	100 µL
Total volume	10 mL

Load cells

2.2 Remove growth medium from the cells and add 20 µL (for a 384-well plate) or 80 µL (for a 96-well plate) of 1X Loading Buffer (Step 2.1) to each well.

2.3 Incubate the cells at 37°C for 60 minutes in a benchtop incubator for cell culture **not** infused with 5% CO₂. During this time, prepare the Assay Buffer and the Stimulus Buffer.

Note: Optimal loading times and incubation temperatures vary for different cell types.

Prepare Assay Buffer

2.4 Prepare 10 mL of Assay Buffer containing FluxOR™ Background Suppressor:

Deionized water	7.9 mL
FluxOR™ Assay Buffer, 10X (Component C)	1 mL
FluxOR™ Background Suppressor (Component I)	1 mL
Probenecid, reconstituted in deionized water (Step 1.1)	100 µL
Total volume	10 mL

Prepare Stimulus Buffer

2.5 Prepare 10 mL of Stimulus Buffer:

- For voltage-gated channels, prepare the **High Potassium Stimulus Buffer**.
- For non-voltage-gated channels, prepare the **Basal Potassium Stimulus Buffer**.

IMPORTANT! The concentrations of potassium and thallium recommended in the following buffers are starting points for further optimization. We recommend that you prepare and test a matrix of varying potassium and thallium concentrations in Stimulus Buffer and choose the concentrations that result in optimal performance and pharmacology for each target and cell type.

High Potassium Stimulus Buffer (for voltage-gated channels)

This 5X formulation injects an elevated level of potassium to depolarize the cells and open voltage gated (Kv type) potassium ion channels. Prepared as below, this stimulus buffer elevates extracellular potassium by 10 mM and adds 2 mM thallium (final) to the solution.

Chloride-free Stimulus Buffer (Component F)	2 mL
Potassium Sulfate (K_2SO_4) (Component G)	2 mL
Thallium Sulfate (Tl_2SO_4) (Component H)	1 mL
Deionized water	5 mL
Total volume	10 mL

Basal Potassium Stimulus Buffer (for non-voltage-gated channels)

This 5X formulation injects the thallium stimulus in an isotonic solution to a final concentration of 2 mM to measure the activity of tonically open “resting” or Kir type potassium ion channels. Prepared as below, this stimulus buffer adds 2 mM thallium (final) to the solution, with no additional potassium.

Chloride-free Stimulus Buffer (Component F)	2 mL
Thallium Sulfate (Tl_2SO_4) (Component H)	1 mL
Deionized water	7 mL
Total volume	10 mL

Perform assay

- 2.6 Remove the Loading Buffer and replace it with 20 μ L/well (for a 384-well plate) or 80 μ L/well (for a 96-well plate) of Assay Buffer containing FluxOR™ Background Suppressor (from Step 2.4) in each well.
- 2.7 *Optional:* Add the test compounds and incubate for 10–30 minutes at 18–24°C.
- 2.8 Perform the FluxOR™ Red assay using a kinetic dispense microplate reader:
 - a. Set up the instrument with standard TRITC filters or set the excitation wavelength to 540–560 nm and the emission wavelength to 580–600 nm.
 - b. After 10 seconds of recording, add Potassium Channel Stimulus buffer to the cells at a 1:5 final dilution (5 μ L/well for a 384-well plate or 20 μ L/well for a 96-well plate).
 - c. Read the plate every 1–2 seconds for 1–3 minutes.

Using high thallium concentrations to detect difficult targets

When using high thallium concentrations to detect difficult targets, replace the FluxOR™ Assay Buffer (Component C) in the working assay buffer solution (Step 2.4) with Chloride-free Stimulus Buffer (Component F) after the cell loading procedure.

To prepare the working assay buffer, mix 2 mL of Chloride-free Stimulus Buffer (Component F) with 8 mL of deionized water and 100 µL of Probenecid (Component E). These conditions may help in obtaining signals from poorly expressing targets or targets with limited thallium permeability.

Sufficient Chloride-free Stimulus Buffer (Component F) and Thallium Sulfate (Tl₂SO₄) Concentrate (Component H) are included in the kit to run the assay under high-thallium conditions, allowing up to 8 mM free thallium after dilution into the microtiter plate.

Appendix: Assay principle

The FluxOR™ Red Reagent (Component A) is a fluorogenic indicator dye, which is loaded into cells as a membrane-permeable AM ester (Figure 1, page 2). Before loading into the cells, the FluxOR™ Red Reagent is dissolved in DMSO (Component B) and further diluted with FluxOR™ Assay Buffer (Component C). Loading is assisted by the proprietary PowerLoad™ concentrate (Component D), 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™ Red 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 E), which blocks organic anion pumps.

For most applications, cells are loaded with the dye at 37°C, but this varies markedly with cell type. For best results, the dye-loading buffer is removed and replaced with fresh, dye-free FluxOR™ Assay Buffer (Component C) containing Probenecid and FluxOR™ Background Suppressor (Component I) before the HTS assay. If you wish to run the assay in the absence of the background suppressor, a second wash step may be necessary to remove residual dye from the solution that can mask the response from the cells.

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™ Red dye exhibits a strong increase in fluorescence intensity at its peak emission of 590 nm. Baseline and stimulated fluorescence is monitored in real time to give a dynamic, functional readout of thallium redistribution across the membrane.

The FluxOR™ Red assay may also be used to study enzymatic potassium transport processes that accommodate the transport of thallium into cells, while voltage-gated potassium channels are opened by the co-administration of potassium and thallium in the stimulus buffer. Resting and inward rectifier potassium channels such as Kir1.1 and Kir2.1 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 formulation of the FluxOR™ Red Reagent allows its use 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. 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™ Red 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™ Red Reagent in FluxOR™ Assay Buffer, which is removed and replaced with 1X chloride-free buffer containing Probenecid prior to running the assay.

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™ Red dye should be readily visible with a TRITC 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: Incomplete removal of solutions 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.

Ordering information

Cat. no.	Product Name	Unit Size
F20018	FluxOR™ Red Potassium Ion Channel Assay *for 2 microplates*	1 kit
F20019	FluxOR™ Red Potassium Ion Channel Assay *for 10 microplates*	1 kit
Related Products		
F20015	FluxOR™ Green Potassium Ion Channel Assay *for 2 microplates*	1 kit
F20016	FluxOR™ Green Potassium Ion Channel Assay *for 10 microplates*	1 kit
F20017	FluxOR™ Green Potassium Ion Channel Assay *for 100 microplates*	1 kit
P10020	PowerLoad™ concentrate, 100X	5 mL
P36400	Probenecid, water soluble	10 × 77 mg
A14291DJ	Live Cell Imaging Solution, 1X	500 mL
B10512	BackDrop™ Background Suppressor for Microplate Applications, 10X	5 mL
B10513	BackDrop™ Background Suppressor for Microplate Applications, 10X	50 mL

Documentation and support

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.

Obtaining support

For the latest services and support information for all locations, go to thermofisher.com/support.

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- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (thermofisher.com/support)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS

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Revision history: Pub. No. MAN0016213

Revision	Date	Description
A.0	September 2016	New document

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