Next-generation detection of potassium ion flux

FluxOR II Green Potassium Ion Channel Assay.

Potassium channels are ion-selective protein pores that span the cell's plasma membrane and serve to establish and regulate membrane potential. In excitable cells such as neurons and myocytes, these channels function both to shape the action potential and to reset the cell's resting membrane potential. The Invitrogen™ FluxOR™ II Green Potassium Ion Channel Assay is the newest tool for high-throughput detection of potassium ion channel and transporter activities.

Similar to the first-generation FluxOR assay [1,2], the FluxOR II Green Potassium Ion Channel Assay is a homogeneous fluorescence-based microplate assay designed for high-throughput screening (HTS) measurements of potassium channel activity. The assay takes advantage of both the well-established permeability of potassium channels to thallium ions and a highly sensitive fluorogenic thallium indicator, the FluxOR II Green reagent (Figure 1). The fluorescent signal reported in this assay serves as a surrogate readout of the activity of any ion channel or transporter that is permeable to thallium, including hERG, Kv1.3, Kir2.1, KATP, and other pharmacologically important potassium channels from all branches of this large gene family.

FluxOR II Green in action

The FluxOR II Green Potassium Ion Channel Assay is easy to use and is compatible with both stably and transiently expressed potassium channels and transporters. The cell-permeant, fluorogenic FluxOR II Green reagent is simply dissolved in DMSO and added to the cells in a loading buffer (prepared with kit components, including Invitrogen™ PowerLoad™ Concentrate). Once inside the cell, the nonfluorescent AM ester on the FluxOR II Green dye is cleaved by endogenous esterases to yield the cell-impermeant thallium-sensitive indicator, which is retained in the cytosol; its extrusion is inhibited by a water-soluble formulation of probenecid, which is included in the loading buffer to block organic anion transporters in the cell membrane.

For detection of voltage-gated ion channels, cells preloaded with the FluxOR II Green reagent are stimulated with an extracellular solution that contains thallium ions (and optionally potassium ions) to depolarize the cells. Upon addition of this stimulus buffer, the extracellular thallium flows down its concentration gradient into the cells and binds to the indicator dye, which emits a fluorescent signal proportional to the number of open channels. Potassium channel or transporter activity is detected by measuring the increase in FluxOR II Green fluorescence (Ex/Em = 495/525 nm) using standard FITC filters. In this way, the fluorescence observed using the FluxOR II Green assay is a quantitative indicator of any ion channel activity or transport process that allows thallium into cells.

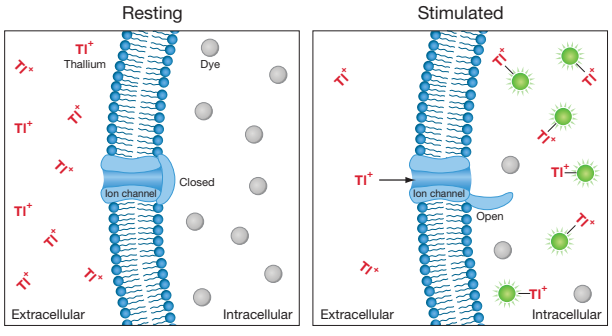

Figure 1. Mechanism of action for the FluxOR II Green Potassium Ion Channel Assay. Basal fluorescence from cells loaded with Invitrogen™ FluxOR™ II Green dye is low when potassium channels remain unstimulated, as shown in the left panel. When thallium is added to the assay with the stimulus, the thallium flows down its concentration gradient into the cells, activating the dye as shown in the right panel.

Advances in the detection of potassium ion flux

Through a multi-tiered approach, we have significantly enhanced the detection of potassium channels with the development of the new-and-improved FluxOR II Green assay. First, the original thallium-sensitive FluxOR dye was modified to dramatically lower the resting background fluorescence before stimulation. The large reduction in background fluorescence exhibited by the FluxOR II Green dye produces a larger assay signal window while greatly reducing stray fluorescence from unincorporated dye. Additionally, an optional background suppressor has been included in the kit to reduce off-target fluorescence from cell culture medium and other extracellular sources. To demonstrate the increased sensitivity achieved with the assay enhancements, we compared the performance of the FluxOR II Green Potassium Ion Channel Assay with that of the original FluxOR assay and the Molecular Devices FLIPR™ Potassium Assay Kit. Using CHO cells stimulated with a mixture of potassium and thallium ions to activate voltage-gated hERG potassium channels, we found that the signal-to-noise ratios (S/N) generated by the FluxOR II Green assay were significantly larger

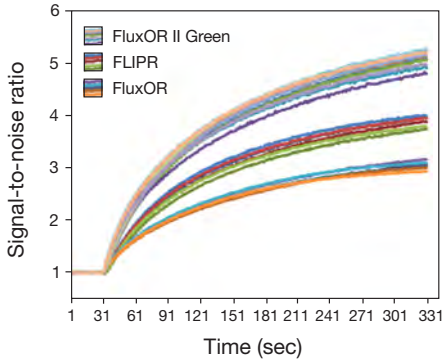

Figure 2. Increased signal-to-noise ratio for the FluxOR II Green Potassium Ion Channel Assay. CHO cells were preloaded with the FluxOR II Green reagent, stimulated with a solution containing 2 mM thallium and 10 mM potassium to stimulate voltage-gated potassium channels, and then analyzed using a Hamamatsu™ FDSS6000 imaging-based plate reader. The other two assays were carried out similarly, according to their supplied protocols. The Invitrogen™ FluxOR™ II Green Potassium Ion Channel Assay (Cat. No. F20016) exhibited a >40% larger signal-to-noise ratio (S/N) as compared with the Molecular Devices FLIPR™ Potassium Channel Assay Kit, and a >100% larger S/N as compared with the original FluxOR Potassium Ion Channel Assay.

than those of the other two assays throughout the time course of the experiment (Figure 2).

The FluxOR II Green assay also displays an improved dynamic range, allowing potassium channels to be detected over a wide range of concentrations and activities. Figure 3 shows the fluorescence detected in CHO cells that were preincubated with different concentrations of E-4031, a hERG-specific blocker, prior to assaying potassium channel activity. As compared with the FLIPR assay and the original FluxOR assay, the FluxOR II Green assay produces larger fluorescent signals throughout the range of inhibitor concentrations tested. The improved assay sensitivity and signal window makes the FluxOR II Green assay more effective at measuring potassium flux over a wider array of channel activities and stimulus and inhibitor concentrations. In these experiments, the FluxOR II Green, FLIPR, and FluxOR assays produced EC_{50} values that correlated well with published values.

Furthermore, we optimized the assay protocol and reagents through an iterative process that resulted in buffer stabilization and more flexibility in experimental design. In addition to the typical wash format, the FluxOR II Green assay can now be performed in a no-wash format, helping to reduce well-to-well variability by eliminating wash steps and media manipulations. The no-wash format also provides a means of assaying pharmacological efficacy using serum-shift assays.

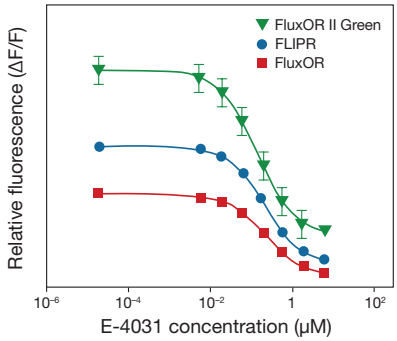

Figure 3. Increased dynamic range of the FluxOR II Green Potassium Ion Channel Assay. CHO cells were preloaded with the FluxOR II Green reagent and stimulated with a solution containing 2 mM thallium and 10 mM potassium to activate voltage-gated potassium channels. Cells were then exposed to different concentrations of the potassium channel blocker E-4031 and analyzed using a Hamamatsu™ FDSS6000 imaging-based plate reader, according to the protocol provided with the Invitrogen™ FluxOR™ II Green Potassium Ion Channel Assay (Cat. No. F20016); the other two assays were carried out similarly, according to their supplied protocols. The FluxOR II Green assay displays an improved detection sensitivity and signal window as compared with the first-generation FluxOR assay or the Molecular Devices FLIPR™ assay, facilitating the analysis of a wider range of potassium channels, including low-expressed or weakly conducting channels.

Learn more about the FluxOR II Green assay

The FluxOR II Green Potassium Ion Channel Assay—available in three different sizes—provides a concentrated thallium solution and all necessary buffers and loading reagents, as well as a detailed protocol for fluorescence detection of potassium channel activity in a homogeneous HTS format. The FluxOR II Green assay has been validated in cells expressing potassium channels either stably or transiently and in 96-, 384-, and 1,536-well plate formats. Learn more about the FluxOR II Green assay and its compatibility with a range of potassium channels and transporters at thermofisher.com/fluxorbp74. ■

References

1. Titus SA, Beacham D, Shahane SA et al. (2009) *Anal Biochem* 394:30–38.
2. Beacham DW, Blackmer T, O'Grady M et al. (2010) *J Biomol Screen* 15:441–446.

Product	Quantity	Cat. No.
FluxOR™ II Green Potassium Ion Channel Assay	2 microplates	F20015
	10 microplates	F20016
	100 microplates	F20017