FluxOR™ Potassium Ion Channel Assay

Catalog Number F10016, F10017

Doc. Part No. 100007420 **Pub. no.** MAN0002485 **Rev.** A.0

Detailed protocol is available online at **www.thermofisher.com.**

Protocol summary

1. For each microplate, prepare 10 mL of **Loading Buffer**:

PowerLoad™ Concentrate	100 µL
FluxOR™ Reagent, reconstituted in DMSO	10 µL
Deionized water	8.8 mL
FluxOR™ Assay Buffer	1 mL
Probenecid, reconstitued in deionized water	100 µL
Total volume	10 mL

2. Remove media from cells and add 20 µL (for 384-well plate) or 80 µL (for 96-well plate) of **Loading Buffer** to each well.

3. Incubate for 60 minutes at 18–24°C, protected from direct light. During incubation, prepare the **Assay** and **Stimulus Buffers**.

4. Prepare 10 mL of **Assay Buffer:**

Deionized water	8.9 mL
FluxOR™ Assay Buffer	1 mL
Probenecid, reconstitued in deionized water	100 µL
Total volume	10 mL

Protocol continued on reverse side

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

ThermoFisher SCIENTIFIC

5. Prepare 5 mL of **Stimulus Buffer:***

	$+K^+$	$-K^+$
Deionized water	2.5 mL	3.5 mL
FluxOR™ Chloride-free Buffer	1 mL	1 mL
K_2SO_4 Concentrate	1 mL	—
Tl_2SO_4 Concentrate	0.5 mL	0.5 mL
Total volume	5 mL	5 mL

* Additional Stimulus Buffer may be required based on liquid handling capabilities.

6. Remove **Loading Buffer** and replace with 20 µL per well (for 384-well plate) or 80 µL per well (for 96-well plate) of **Assay Buffer**.

7. *Optional*: Add the test compounds, then incubate for 10–30 minutes at 18–24°C.

8. Perform assay using a fluorescent plate reader with liquid handling features.

- Use a FITC filter or set the excitation/emission wavelengths to ~490 nm/~525 nm.
- Add **Stimulus Buffer** after 10 seconds of recording (5 µL for 384-well plate or 20 µL for 96-well plate).
- Read plate every 1–2 seconds for 1–3 minutes.

For more information, visit **www.thermofisher.com/fluxor**.

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

For support visit **thermofisher.com/support**

thermofisher.com

30 June 2016

ThermoFisher SCIENTIFIC