

FluxOR™ Potassium Ion Channel Assay (Cat. no. F10016, F10017)

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, reconstituted in dH ₂ O | 100 µL |
| <hr/> | |
| 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, reconstituted in dH ₂ O | 100 µL |
| <hr/> | |
| Total Volume | 10 mL |

Protocol continued on reverse side

FluxOR™ Potassium Ion Channel Assay

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 ₂ SO ₄ Concentrate | 1 mL | – |
| Tl ₂ SO ₄ Concentrate | 0.5 mL | 0.5 mL |
| <hr/> | | |
| 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 test compounds, and 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.invitrogen.com/fluxor.

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