

TEFLabs' Thallos™ ¹ No Wash cell-based assays for Potassium flux provides a method for monitoring thallium flux in cells expressing thallium permeable ion channels or transporters. Cells may natively express the ion channels, or the ion channels may be introduced into the cells using well-known transfection and transduction techniques.

Thallos™, is bright, sensitive, rapidly loading, and produces superior signal-to-background readings compared to FluxOR™ ² using a No Wash format. TEFLabs' Thallos™ No Wash cell-based assays offer users outstanding performance suitable for the rapid and effective support of assay development, hit identification, hits-to-leads and probe/lead development activities in both academic and industrial settings..

TEFLabs' Thallos™ No Wash Assay Kits provide an easy to follow protocol and include the buffers necessary for dye loading and compound addition. Water-soluble probenecid is provided to inhibit the cell's organic anion transporters from pumping the indicator outside of cells into extracellular medium. Optimized for use in No Wash format users may decide to add a quencher dye to further suppress background fluorescence. The cells may, optionally, be washed after the loading buffer solution is provided to the cells.

TEFLabs offers users a choice between two buffer systems, a Sodium Gluconate Stimulus Solution and a Potassium Gluconate Stimulus Solution to further optimize the assay for target-specific applications. TEFLabs offers an explorer kit option that provides enough of each buffer solution for users to test in their application. Please contact us to speak to a representative about custom kit configuration or replacing your current Potassium flux assay with our easy to use and cost-effective TEFLabs' Thallos™ No Wash Assay Kits.

¹ Thallos is a trademark of Vanderbilt University. Patent-Pending

² FluxOr is a trademark of LifeTechnologies

TEFLabs' Thallos™ No Wash Potassium Channel Assay

Component	Material	10 x 1 plates explorer (#0910)	10 x 1 plates (#0912, #0913)	1 x 10 plates (#0914, #0915)	10 x 10 plates (#0916, #0917)
A	Reagent-Thallos	10 x 25 ug vials	10 x 25 ug vials	1 x 250 ug	10 x 250 ug
B	7% wt/v Pluronic F-127 in anhydrous DMSO	10 x 50 uL	10 x 50 uL	1 x 500 uL	10 x 500 uL
C	Probenecid, water-soluble salt	10 x 15 mg	10 x 15 mg	1 x 150 mg	10 x 150 mg
D	HHBSS	1 x 500 mL	1 x 500ml	1 x 500 ml	3 x 1000 ml
E	Thallium sulfate	1 x 40 mL 50 mM Tl ₂ SO ₄	1 x 40 mL 50 mM Tl ₂ SO ₄	1 x 40 mL 50 mM Tl ₂ SO ₄	10 x 40 mL 50 mM Tl ₂ SO ₄
F	Stimulus buffer 1* (Sodium Gluconate)	1 x 75 mL	10 x 7.5 mL (#0912)	1 x 75 mL (#0914)	10 x 75 mL (#0916)
G	Stimulus buffer 2* (Potassium Gluconate)	1 x 75 mL	10 x 7.5 mL (#0913)	1 x 75 mL (#0915)	10 x 75 mL (#0917)

*assay kits will have either Sodium Gluconate Stimulus Buffer or Potassium Gluconate Stimulus Buffer, explorer kits will contain both.

Stimulus Buffer 1	Stimulus Buffer 2
Sodium Gluconate Stimulus Buffer	Potassium Gluconate Stimulus Buffer
Sodium Gluconate: 276mM	Sodium Gluconate: 276mM
Calcium Sulfate: 2.6 mM	Calcium Sulfate: 2.6 mM
Magnesium Sulfate: 1.6 mM	Magnesium Sulfate: 1.6 mM
D-glucose: 11.2 mM	D-glucose: 11.2 mM
HEPES, pH 7.3: 40 mM	HEPES, pH 7.3: 40 mM

Storage and Handling

- component A:** Thallos™ Reagent. Store at -20°C. Protect from light and avoid repeated freeze and thawing cycles.
- component B:** 7% wt/v Pluronic F-127 in anhydrous DMSO. Protect from light and avoid repeated freeze and thawing cycles.
- component C:** Probenecid, water-soluble salt. Protect from light and avoid repeated freeze and thawing cycles.
- component D:** HHBSS buffer: Sterile filtered solution, pH= 7.3 +/-0.2. Keep refrigerated after opening(4°C).
- component E:** Thallium sulfate is toxic. Use caution, proper personal protection equipment, and properly dispose any waste. Keep at room temperature, refrigerated after opening (4°C)
- component F:** Sodium Gluconate Stimulus Buffer. Keep refrigerated Keep refrigerated after opening(4°C).
- component G:** Potassium Gluconate Stimulus Buffer. Keep refrigerated Keep refrigerated after opening(4°C).

Shelf Life: Valid for six months after delivery, if stored properly.

Notes

Both Component F and Component G are provided in Cat. Number 0910 in order for customers to determine which stimulus buffer is most appropriate for their application. We recommend testing 5 plates with each buffer type.

End-users should empirically determine the desired amount of Thallium sulfate

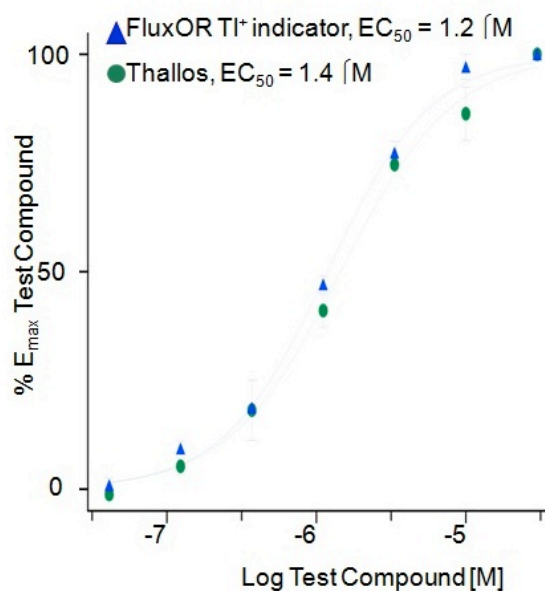
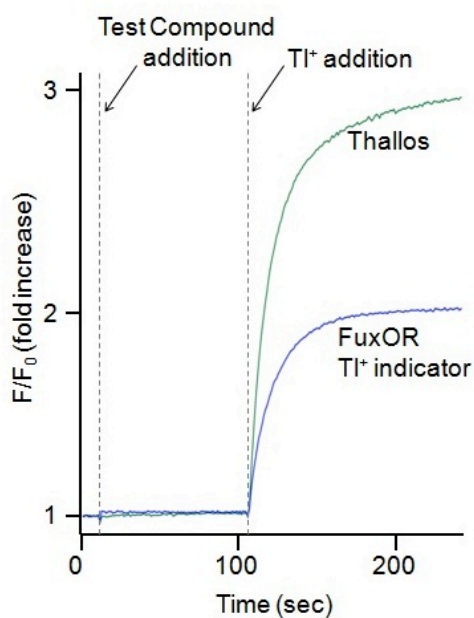
SAMPLE PROTOCOL For #0910, #0912, #0913(makes solution for 1 plate):

1. Add 30 μ L 7% wt/v Pluronic F-127 in anhydrous DMSO (Component B) into 25 μ g Thallos™ (Component A), vortex to mix thoroughly.
2. Dissolve 15 mg water-soluble Probenecid (Component C) into 10 mL HHBS buffer (Component D), vortex to mix thoroughly.
3. Combine 25 μ L Thallos/pluronic solution (step 1) with assay buffer/probenecid/solution (step 2), vortex gently to avoid foaming.
4. Remove cell medium and replace with 100 μ l (96-well plate) or 20 μ l (384-well plate) of dye loading solution (step 3) into each well.
5. Incubate for 1 hour at room temperature. Incubation time and temperature may be varied for optimal results.
6. Add Thallium sulfate solution(Component E) to Gluconate Stimulus Buffer (component F or G) to make Thallium Stimulus Buffer. For Example, 5mL Gluconate Stimulus Buffer (component F or G), 1 mL Thallium sulfate solution(Component E), 4mL water. Vortex to mix.
7. Prepare test compounds in HHBS (Component D) or Thallium Stimulus Buffer as appropriate.
8. Monitor the fluorescence intensity (Ex/Em= 490nm/515nm) with the addition of test compounds and thallium stimulus buffer, as desired, on appropriate fluorescence plate reader.

SAMPLE PROTOCOL For #0914, #0915, #0916, #0917

(makes solution for 10 plates)

1. Add 300 μ L 7% wt/v Pluronic F-127 in anhydrous DMSO (Component B) into 250 μ g Thallos™ (Component A), vortex to mix thoroughly.
2. Dissolve 150 mg water-soluble Probenecid (Component C) into 100 mL HHBS buffer (Component D), vortex to mix thoroughly.
3. Combine 250 μ L Thallos/pluronic solution (step 1) with assay buffer/probenecid/solution (step 2), vortex gently to avoid foaming.
4. Remove cell medium and replace with 100 μ L (96-well plate) or 20 μ L (384-well plate) of dye loading solution (step 3) into each well.
5. Incubate for 1 hour at room temperature. Incubation time and temperature may be varied for optimal results.
6. Add Thallium sulfate solution(Component E) to Gluconate Stimulus Buffer (component F or G) to make Thallium Stimulus Buffer. For Example, 50mL Gluconate Stimulus Buffer (component F or G), 10 mL Thallium sulfate solution(Component E), 40 mL water. Vortex to mix.
7. Prepare test compounds in HHBS (Component D) or Thallium Stimulus Buffer as appropriate.
8. Monitor the fluorescence intensity (Ex/Em= 490nm/515nm) with the addition of test compounds and thallium stimulus buffer, as desired, on appropriate fluorescence plate reader.



Using “no wash” format Thallos produces a higher fold increase in fluorescence compared to FluxOR TI+ indicator. Both dyes produced excellent Z' (Thallos™ = 0.87, FluxOR™ TI+ indicator = 0.68) in this assay and nearly identical potencies for compounds tested.³

³Image and description courtesy of Dave Weaver, Vanderbilt University. Studies performed in mammalian potassium channel.