# CELT-503 General Protocol

## REAGENTS

- **WASH BUFFER*:**
  - Tris-HCl or HEPES (pH 7.4)
- **INCUBATION BUFFER*:**
  - Tris-HCl or HEPES (pH 7.4), $MgCl_2$, NaCl, GDP, Saponin
- **MEMBRANE (10x)***
- **COMPOUNDS/CONTROL (10x)***
- **LIGAND (10x): CELT-503 (Fluorescent GTPγS)**
  - Recommended Final Concentration: 100nM
    - Intermediate Aliquot: 1µM in Incubation Buffer

## ASSAY CONDITIONS

- Final Volume: 250µL/well
- Incubation* in Incubation Plate with shaking at 25°C

## ASSAY PROTOCOL

1. Add 175µL/well of Incubation Buffer to the Incubation Plate
2. Add 25µL/well of Membrane
3. Add 25µL/well of Compounds/Control
4. Incubate for 15 minutes
5. Add 25µL/well of CELT-503
6. Incubate for 1 hour
7. Transfer 200µL to the Filter Plate
8. Filter
9. Wash 3 times with 200µL/well of Wash Buffer
10. Read at λEx: 589 – λEm: 616

***Optimize concentrations and incubation conditions for each receptor (GPCR) for optimal results***

**For more details on the setup of assays using GTPγS, you can consult the following protocol:**
https://resources.perkinelmer.com/corporate/content/relatedmaterials/posters/sps_trfbasedgtpbindingassay.pdf