## *GTPγS assay (SPA method) - Sample protocol*

ES-010-M membranes (Adenosine A1 receptor)
**Please note that buffer composition, concentration of membrane, concentration of GDP, and concentration of beads will need to be optimized for different receptors.*

### Reagent preparation

- Assay buffer 20 mM HEPES pH 7.4; 100 mM NaCl, 10 µg/ml saponin, 1 mM MgCl2
- Membranes diluted in assay buffer to give 250 µg/ml (2.5 µg/10 µl), keep on ice.
- GDP make a 100 µM solution in assay buffer (final concentration of 10 µM)
- Beads PVT-WGA (PerkinElmer, RPNQ001), dilute in assay buffer at 50 mg/ml (0.5 mg/10 µl)
- GTPγ $^{35}$S (PerkinElmer, NEG030X), diluted in assay buffer to give ~25.000 dpm/10µl
- Ligands CPA (Sigma, C-8031), CCPA (Tocris, 1705), GR 79236 (Tocris, 1957), DPCPX (Phoenix, 439) and 1,3-dipropyl-8-phenylxanthine (Tocris, 486); all diluted in assay buffer
- 96 well plate Optiplate™ (PerkinElmer, 6005299)
- Reader TopCount® (PerkinElmer)

### Make master mixes

1. Mix the membranes and GDP (volume:volume) and incubate for at least 15 min. on ice
2. Mix the GTPγ-$^{35}$S and the beads (volume:volume) just before starting the reaction

### Add reagents to OptiPlate

Add sequentially in the order indicated:

3. 50 µl of ligand
4. 20 µl of the membranes:GDP mix
5. 10 µl of buffer*
6. 20 µl of the GTPγ $^{35}$S:beads mix

*For antagonists testing, 10 µl of the reference agonist (CPA) are added, at a concentration corresponding to the EC80 (9.00 nM), instead of 10 µl of buffer.

### Incubation and count

7. Cover the plate with a TopSeal-A™
8. Shake on an orbital shaker for 2 min
9. Incubate for 1h at RT
10. Centrifuge for 10 min. at 2000 rpm
11. Incubate for 1h at RT
12. Count for 1 min