MANUAL

Cell transient transfection with tagged GPCR or RTK plasmids

Revvity proposes a broad list of GPCR and RTK plasmids encoding SNAP-tag®, CLIP-tag® and HaloTag® at the N-terminal position Once transfected, cells expressing the tagged receptor can be labeled with the specific substrate derivatized with HTRF fluorophores.

Material

Plasmids

- The list of GPCR and TRK plasmids (human) is available on our website.

Recommended reagents

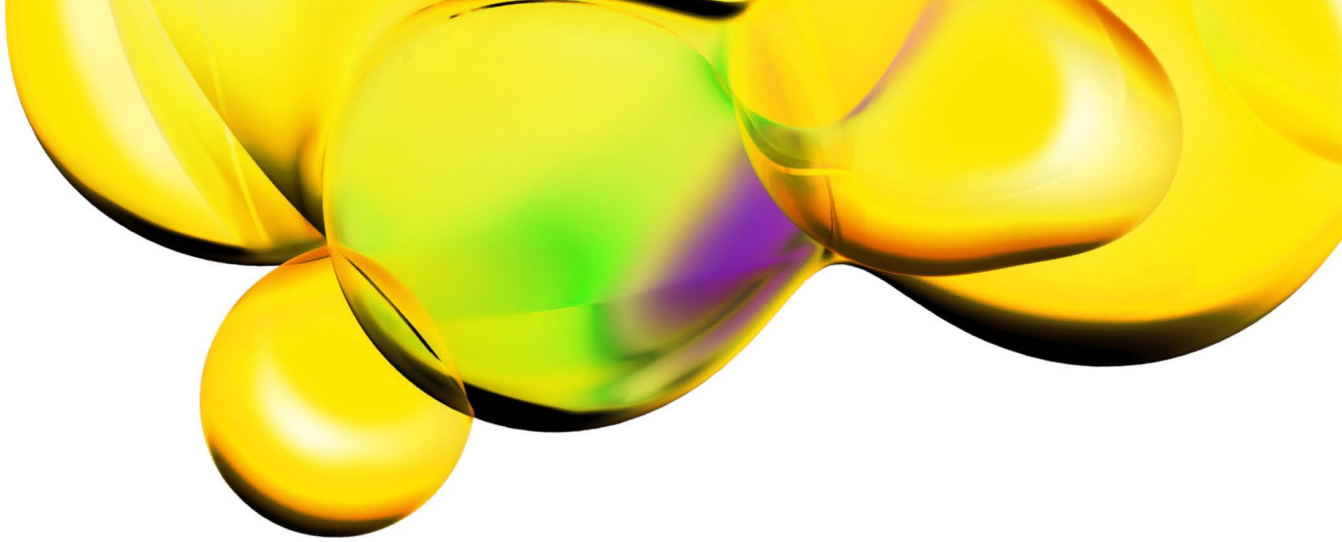
- HEK 293 cells
- PBS (Life technologies Cat.# 10010-031)
- Cell dissociation buffer (Sigma Cat.#C5914)
- Opti-MEM, with GlutaMAX and phenol red (Life technologies Cat.# 51985-026)
- Lipofectamine 2000 (Life technologies Cat.#11668-027)
- Culture medium for HEK293: DMEM (Life technologies Cat.#31966-021) supplemented with Penicillin-Streptomycin 1X (Life technologies Cat.# 15070-063), HEPES 2mM (Life technologies Cat.#15630-122), MEM 1X (Life Technologies Cat.#11140-035)

Transient transfection - flask T175

Two to three days before the transfection, seed the cells in a T175 flask. For an efficient transfection, cells should be adherent with a confluency around 70 - 80%

1. Prepare the transfection mix, add in the following order
   - Opti-NEM: 8 mL
   - Lilofectamine: 60 µL
   - Plasmid (1µg/µL): 20 µL

   Incubate for 20 minutes at RT
2. Gently remove the cell culture medium by aspiration
3. Wash cells with 5 mL of PBS
4. Add the 8.08 mL of transfection mix preparation then add 12 mL of cell culture medium
5. Incubate for 1 night at 37°C + 5% CO2.
6. Cells expresing the substrate-tag receptor can now be labelled with Tag-lite SNAP-Lumi4-Tb and used to carry out a binding assay



Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

www.revvity.com

Revvity, Inc.
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com