Seeding cells in LigandTracer polydopamine coated MultiDish 2×2

This protocol describes the preparation of LigandTracer *polydopamine coated* MultiDish 2×2 with adherent or semi-adherent cells to obtain one target area and one reference area in each compartment, which is suitable for measurements in LigandTracer.

Important information

The cell dish should be prepared at least two days prior to measurement for best results.
It is highly recommended to always perform cell culture work in a sterile environment.

Materials

- LigandTracer *polydopamine coated* MultiDish 2×2* (Cat. No. 1-4-203, Ridgeview Instruments AB)
- Adherent or semi-adherent cells
- Cell culture medium
- Phosphate-buffered saline (PBS)
- Trypsin or equivalent

* Note that LigandTracer MultiDish is only compatible with LigandTracer Green Second generation (serial numbers RCF-041XXX) or older LigandTracer Green instruments that have been upgraded.

Procedure

1. Re-suspend the adherent cells in fresh cell culture medium using trypsin or an equivalent solution.
2. Centrifuge the cell solution to obtain a cell pellet. Remove the supernatant and add PBS to reach a cell concentration of approximately 0.5-1×10^6 cells/ml for measurements two days later.
3. Position the dish on the LigandTracer MultiDish 2×2 template and ensure correct alignment. Carefully dispense 600 µl of the cell solution over the slightly darker circular areas in sectors A and C, as indicated by the template. The polydopamine coated areas in B and D will be used for reference subtraction and can either be cell free (standard approach) or contain a negative cell line.
4. Incubate the dish at room temperature for 30-60 min.
5. Carefully remove the remaining cell solution once the cells have attached. Add approximately 5 ml cell medium to each compartment. It is advised to work with medium that contains fetal bovine serum or a similar protein cocktail to reduce the risk of non-specific ligand binding during the measurement.
6. Keep the dish in an incubator until measurement in LigandTracer, preferably for at least two days to enhance adherence.
7. Confirm under a microscope that the cells have attached only where intended. Remove with a cell scraper if you find more than a few scattered cells in areas far from where the cells were seeded.
8. Choose a pre-defined MultiDish template in LigandTracer Control that provides suitable settings for the experiment, and start the measurement. The detection time can be altered by unlocking the template.