LigandTracer

Protocol

Seeding cells in polydopamine coated dishes

This protocol describes the preparation of *polydopamine coated* LigandTracer MultiDish 2×2 or non-treated Petri dishes with adherent or semi-adherent cells for LigandTracer experiments.

Important Information

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

Materials

Polydopamine coated dish[1], 87-89 mm (diameter) and about 15 mm (height), e.g. polydopamine coated MultiDish 2x2 *for coatings** (Cat. No. 1-4-204, Ridgeview Instruments AB) or non-treated polystyrene Nunc Petri Dish (Cat. No. 263991, Thermo Fisher Scientific).

- Adherent or semi-adherent cells
- Cell culture medium
- Phosphate-buffered saline (PBS)
- Trypsin or equivalent

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. Carefully dispense 600 µl of the cell solution over the slightly darker circular polydopamine coated areas. You can use a coating template to correct positioning of the drops. For a MultiDish, add cell solution in sectors A and C. 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. Similarly, for a regular dish, the reference area can either be cell free or contain a negative cell line, as indicated by the images on the next page.

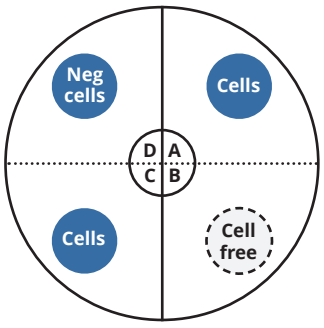
4. Incubate the dish at room temperature for 30-60 min.

5. Carefully remove the remaining cell solution once the cells attached. Add approximately 5 ml cell medium to each compartment (each half) in the MultiDish or 10 ml to a regular dish. 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.

***** LigandTracer MultiDish 2x2 is only compatible with LigandTracer Green Second generation (serial numbers RCF-041XXX) or older LigandTracer Green instruments that have been upgraded.

[1] See protocol *Coat with polydopamine to enhance cell adhesion*

MultiDish 2x2



6. Keep the dish in an incubator until the LigandTracer experiment, preferably for at least two days to enhance adherence.

7. Confirm under a microscope that the cells 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. Replace media with 1.8 ml fresh medium in each compartment (each half) in a MultiDish or 3 ml fresh medium in a regular dish.

8. Choose a pre-defined assay template in LigandTracer Control that provides suitable settings for the experiment, and start the experiment. The detection time can be altered by unlocking the template.

More information can be found in protocols *A typical LigandTracer measurement* and *A typical LigandTracer measurement with MultiDish 2x2*.

Regular dish