LigandTracer

Protocol

Attaching suspension cells for LigandTracer® measurements

This protocol describes the attachment of suspension cells to plastic dishes using the Biocompatible Anchor for Membrane (BAM) molecule. Stable cell adherence has been confirmed for at least 6 h of measurement in LigandTracer for a number of different cell lines.

Important information

- BAM should be aliquoted as powder and be stored at -20 °C in low-bind or siliconized tubes.
- Negative control cells should be included in the dish as a reference, to correct for background signal.
- The LigandTracer measurement should be conducted one or two days (optimal time point is cell dependent) after preparation of the dish. Cell viability and adherence is typically improved by using CO_2-independent media during the assay.
- If using a MultiDish 2x2 for coatings, follow the protocol below but add four drops in step 2, one in each sector A-D. Add target cells in sectors A and C and negative control cells in B and D.

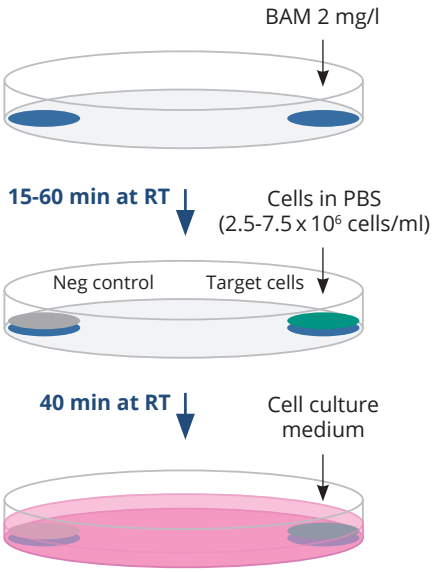
***** LigandTracer MultiDish 2x2 is only compatible with LigandTracer Green Second generation (serial numbers RCF-041XXX) or older LigandTracer Green instruments that have been upgraded.

Materials

- BAM (SUNBRIGHT® OE-040CS, NOF Corporation)
- Dish, 87-89 mm (diameter) and about 15 mm (height), e.g., LigandTracer MultiDish 2×2 *for coatings** (Cat. No. 1-4-204, Ridgeview Instruments AB) or non-treated polystyrene Nunc Petri Dish (Cat. No. 263991, Thermo Fisher Scientific)
- PBS
- Milli-Q water (MQ)
- Cell culture medium with fetal bovine serum (FBS) or similar

Procedure

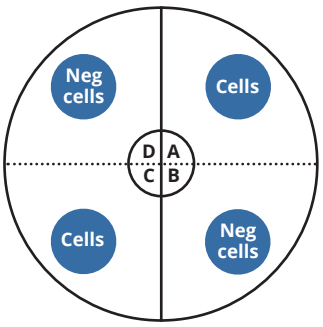
1. Dissolve BAM to 2 mg/ml in MQ (dissolving BAM in PBS might improve attachment for sensitive cells). Estimate usage is 0.8 mg per cell area. Avoid touching the powder with the pipette tip since the powder is highly electrostatic. Vortex for a few minutes until the powder is completely dissolved. The BAM solution is unstable and should be used immediately after it has been solubilized. Discard any excess BAM solution afterwards.
2. Add 2×400 µl BAM solution as two drops of approximately 1 cm in diameter near the rim of the dish, separated 180 degrees. It is important to add the BAM solution slowly and to make sure that the drops do not touch the rim of the dish. A distance of 2-3 mm between the drops and the rim is recommended. Do not move the dish once the BAM solution has been added, until step 6.
3. Incubate for 15-60 min at room temperature. During incubation: Quantify the concentrations of your cell lines. Spin the cells down, carefully remove all cell culture medium and re-suspend the pellets with sterile PBS to obtain a cell concentration of approximately 2.5×10^6 cells/ml. A higher cell concentration of $5\text{-}7.5\times10^6$ cells/ml is recommended for small cells, such as B-cells.
4. Carefully aspirate most of the BAM solution. Avoid extensive touching of



the coated areas with the pipette tip. Add the cell suspensions slowly, 400 µl per area. The cells should be added immediately after removal of BAM.

5. Allow the cells to adhere for 40 min on the bench.
6. Tilt the dish and remove the cell suspensions far from the cell areas. Add 10 ml cell culture medium. The medium should contain at least 1 % FBS or a similar protein cocktail to coat the plastic surface of the dish, to reduce the risk of non-specific binding during the LigandTracer assay.
7. Carefully put the dish in an incubator and leave over-night.
8. Confirm under a microscope that the cells remain stably attached to the dish prior to the LigandTracer measurement.

MultiDish 2x2



Regular dish