

Adsorb protein to a polystyrene dish

This protocol describes how to adsorb target protein to non-treated polystyrene dishes, for the detection of protein-protein interactions in LigandTracer. The dish may either be a LigandTracer *non-treated* MultiDish 2x2 or a regular Petri dish.

Important information

The end result of the adsorption is typically protein dependent and some proteins do not adsorb well or become damaged during the process.

Materials

- Circular non-treated polystyrene dish, 87-89 mm (diameter) and about 15 mm (height), e.g. LigandTracer *non-treated* MultiDish 2x2* (Cat. No. 1-4-202, Ridgeview Instruments AB) or Nunc Petri Dish (Cat. No. 263991, Thermo Fisher Scientific)
- Target protein
- Phosphate-buffered saline (PBS)
- PBS with an irrelevant protein, e.g. 1 % bovine serum albumin (BSA)

* 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. Dissolve the protein in PBS. A concentration of 5-50 µg/ml is often suitable when using antibodies or proteins of similar size.
2. Place the dish horizontally and add the protein solution as 500-750 µl drops near the rim of the dish. Use a coating template to ensure correct positioning of the drops. The number of drops depends on purpose and dish choice. For example, include several target areas to detect the binding of a labeled ligand to different target proteins in one measurement. At least one area should be left target free, for reference subtraction.
3. Let the protein adsorb for at least 2 h at room temperature (RT).
4. Remove excess protein solution and carefully rinse the target area(s) twice with PBS. Avoid rinsing the reference area.
5. Add 3-10 ml PBS containing a high concentration of irrelevant protein (e.g. 1 % BSA) to coat the plastic surface of the dish, which reduces the risk of non-specific binding during the measurement. Leave for at least 1 h at RT.
6. Use the coated dish immediately, or remove the coating solution and store it dry at 4 °C. Storage is often possible for several weeks without losing much of the target activity (protein dependent).
7. Make sure to use LigandTracer instrument settings that are suitable for the dish type and the number of target areas that you have prepared. Choose any of the pre-defined templates in LigandTracer Control or define your own settings by unlocking an opened template.
8. Start the run.

MultiDish 2x2



Regular dish

