RASTRUM

Protocol

AlphaLISA® SureFire® Ultra™ Assay for RASTRUM™ 3D Cell Models

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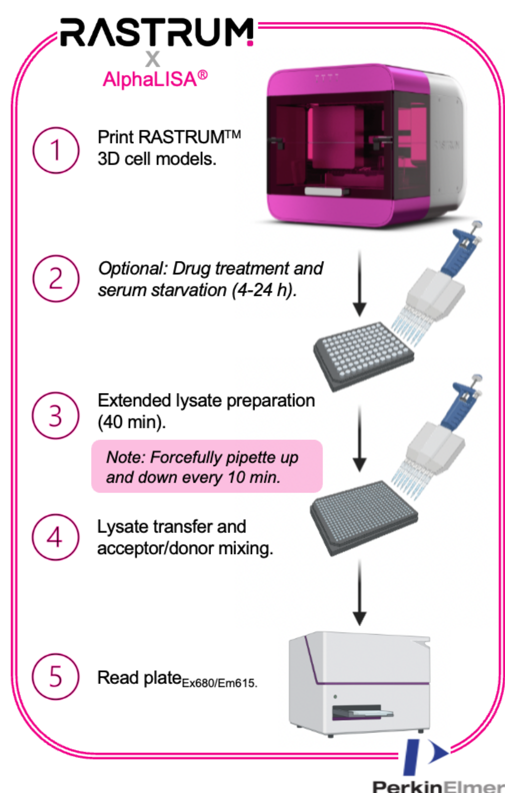
Introduction

Small and large molecule drug discovery projects, particularly high-throughput screening, widely use high-sensitivity protein assays such as Alpha (amplified luminescence proximity homogeneous assay). Alpha is a bead-based assay platform employing oxygen-channelling chemistry for the detection and quantification of various biomolecules. Herein, we describe a protocol for the Alpha assay using RASTRUM 3D cell models.

Equipment and reagents required, but not provided

- RASTRUM 3D cell models
- AlphaLISA® SureFire® Ultra™ kit
    - Lysis Buffer (5X)
    - Activation Buffer
    - Reaction Buffer 1
    - Reaction Buffer 2
    - Dilution Buffer
    - AlphaLISA® CaptSure™ Acceptor Beads (2 mg/mL in PBS plus 0.05 % Proclin-300)
    - Alpha Streptavidin Donor Beads (2 mg/mL in PBS plus 0.05 % Proclin-300)
    - Positive Control Lysate
- Dulbecco's Phosphate-Buffered Saline (DPBS)
- Serum-containing (or serum-free) cell culture medium
- Multichannel pipette
- Alpha Technology-compatible plate reader
- *Optional: serum-containing (or serum-free) cell culture medium, drug compounds*

Graphical Protocol



Protocol

RASTRUM 3D Cell Culture

1. Print RASTRUM 3D cell models using your cells and RASTRUM bioinks according to your RASTRUM Protocol as designed using RASTRUM Cloud. Incubate RASTRUM 3D cell models at 37 °C/5 % CO_2 for 1–7 days.
2. *Optional drug treatment: Depending on the cell type and protein analysed, first establish basal levels of protein expression by performing serum starvation (4–24 h) through the removal of cell culture medium from the RASTRUM 3D cell models and replacing with serum-free medium. Thereafter, replace the cell culture medium with drug prepared in serum-free or serum-containing medium (50 µL and 25 µL for 96- and 384-well plates, respectively) for 4–24 h*

Lysate Preparation

3. Remove the cell culture medium from all wells of the plate by tilting the plate at a 45-degree angle, inserting the tip along the edge of the well to just above the bottom of the well, and aspirating the cell culture medium (**Figure 1**).

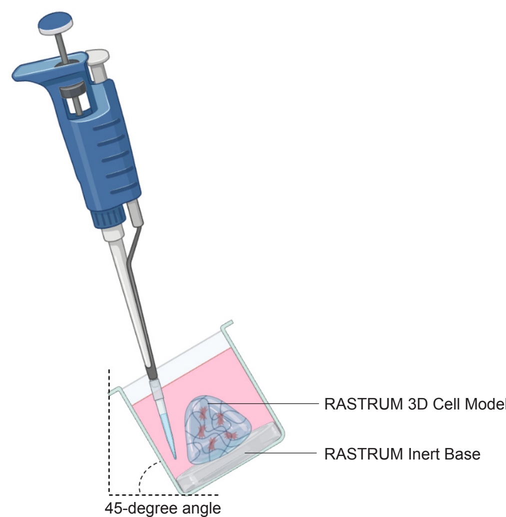


Figure 1. *Schematic representation of liquid handling with RASTRUM 3D cell models.*

4. Wash the RASTRUM 3D cell models by adding 1x DPBS (100 µL and 40 µL for 96- and 384-well plates, respectively) and gently swirling the plate for 5–10 sec.
5. Remove the supernatant using the technique described in Step 3.
6. Lyse the cells within RASTRUM 3D cell models by adding freshly prepared 1X Lysis Buffer (70 µL and 35 µL for 96- and 384-well plates, respectively). Incubate for 40 min at RT.
   Note: Forcefully pipette up and down several times every 10 min.

SureFire Ultra Assay

Note: We recommend following the manufacturer's instructions to detect biomolecules within cell lysates.

7. Take 10 µL of the lysate and transfer to a 384-well Optiplate™ for the assay. Add 10 µL of control lysates to separate wells.
8. Add 5 µL of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film and cover plate with foil. Incubate for 1 h at RT in the dark.
9. Add 5 µL of Donor Mix to wells under subdued light. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 h at RT in the dark.
   Note: Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.
10. Read plate on an Alpha Technology-compatible plate reader, using standard AlphaLISA settings.

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