SEAP Reporter Assay

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Background

- The SEAP reporter assay is used to quantify the activity level of a transcription factor or the expression level of a certain gene. The recognition sequence of a transcription factor or promoter region of a certain gene is transcriptionally fused to a gene encoding secreted embryonic alkaline phosphatase (SEAP). SEAP may then be linked to a reaction with a colorimetric readout to allow simple quantification of transcription factor activity or gene expression.
- This protocol makes use of HEK Blue Detection Media (Invivogen), which allows for the real-time detection of SEAP expression levels in cultured cells.

Equipment and Materials

- Materials
    - DNA to be transfected
    - PEI transfection reagent
    - HEK Blue detection media
    - TLR ligand (e.g. *S. pneumo*, Pam_3CSK_4, LPS, etc.)
    - Spectrophotometer capable to reading Abs at 620nm

Protocol

Day 1/2: Preparing Cells

1. Seed a 6-well plate at a density of 3×10^5 cells per well.
    a. I seed 3ml of cells in media at a density of 1×10^5 cells per 1ml of media.
2. Incubate at 37°C for 24h.
3. After 24h, transfect cells as outlined in the **PEI Transfection** protocol.
    a. I use the following DNA amounts:
        i. SEAP plasmids = 200ng
        ii. TLR plasmids = 60ng
        iii. Scavenger receptor plasmids = 600ng
        iv. Empty vector = up to 1ug

Day 3: Challenging Cells

1. Prepare fresh HEK Blue detection media as needed according to the manufacturer's instructions (media older than 1mo should not be used).
2. Prepare ligands used to induce SEAP activity.
    a. For *S. pneumo*, I use SP P1121 at an MOI of 25 (calculated with original number of cells that were seeded)
    b. For Pam_3CSK_4, I use 1ug/ml
    c. For LPS, I use 10ng/ml
3. Wash cells with PBS.
4. Lift cells in 1.5ml of HEK Blue detection media per well by repeated pipetting or cell lifters. Do not use trypsin or scrape.
    a. The precise volume of detection media to use can vary according to the confluence of the cells. More confluent cells will need a higher volume, etc.
    b. If only a single ligand is to be used, I find it easier to lift cells in detection media already containing the appropriate concentration of ligand.
5. Seed 3-5 wells in a 96-well plate with 200ul of cells
6. Incubate at 37°C.

Day 4: Reading Assay

1. At 24h post-challenge, aliquot media into a clean 96-well plate and read absorbance at 620nm.
2. If no colour change is observed, it is permissible to wait up to 48h before reading.