Mink Lung Cell Assay

Overview: The mink lung cells are stably transfected with luciferase cDNA driven by a portion of PAI-1 promoter. Since PAI-1 is strongly induced by TGFβ on exposure to TGF-β these cells synthesize increased amounts of luciferase, which is measured in a simple assay. The amount of TGF-β in an unknown sample can be determined by comparison to a standard curve using recombinant TGF-β. Since other, mostly unidentified, factors can induce PAI-driven luciferase construct, samples must be tested with and without a specific inhibitor of TGF-β signaling.

1. Plate MLC in a 96-well plate. Generally, we use 35,000 cells/well when a sample of TGF-β in solution is used (for example, a conditioned medium). When MLCs are co-cultured, it is recommended to plate 25,000 MLCs. The number of the other cell type should be determined in a pilot experiment, usually testing 1:1 and 1:2 ratio (MLC:test cells). Freshly trypsinized MLCs are plated in a complete medium (DMEM + 10%FCS) and allowed to attach for 1-2 hours. MLCs are then washed 2X with PBS and then the second cell population is plated in an appropriate medium or a liquid sample is added (conditioned medium or a solution of known concentration of recombinant TGF-β). Generally, every condition is done +/- TGF-β inhibitor (LAP, pan-TGF-β antibody 1D11 or an inhibitor of TGF-βl receptor (SB431542 (Sigma); 5 mM final concentration). Also, each sample is done in a triplicate.

2. Every experiment includes a TGF-β standard curve. We usually test recombinant TGF-β1 in a series of 2X dilutions, from 2ng/ml to 15pg/ml (8 concentrations) along with a medium blank (no TGF-β).

3. Incubate MLC with samples for 16-20 hours.

4. Inspect cells at the end of incubation to confirm that they are still healthy.

5. Wash cells 2X with 200ul of PBS/well. Make sure that after the second wash there is no PBS left.

6. (Ahead of time, prepare adequate volume of lysis buffer. Lysis buffer is bought as a 3X stock from BD Biosciences Pharmingen, Cat: 51-556871. It gets diluted with dH2O).

7. Add 35ul of lysis buffer to each well.

8. Lyse cells for 15 minutes @RT on a shaker. (At this point you can cover samples air-tight with Parafilm and store them at -20C until ready to assay).

9. Get opaque 96-well assay plate. Make sure it is clean! Transfer 30 ul of each sample to the new plate.

10. Ahead of time, make reaction buffer. This is prepared using premade (frozen) aliquots of luciferin, ATP and buffer mix- these aliquots are combined and water added to final volume of 10 ml. Reaction buffer should be @ RT before it is used.

12. After reading is done, flush the tubings with water, then empty the tubings and turn off the machine.

**Buffer stock**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock conc.</th>
<th>Final conc.</th>
<th>1 aliquot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricine</td>
<td>1M (197g/l pH 7.8)</td>
<td>20 mM</td>
<td>200 ul</td>
</tr>
<tr>
<td>Mg(CO)₃Mg(OH)₂5H₂O</td>
<td>21.4 mM (10.4g/l)</td>
<td>1.07 mM</td>
<td>500 ul</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>2.67 M (658g/l)</td>
<td>2.67 mM</td>
<td>10 ul</td>
</tr>
<tr>
<td>EDTA</td>
<td>100 mM</td>
<td>100 uM</td>
<td>10 ul</td>
</tr>
<tr>
<td>DTT</td>
<td>333 mM</td>
<td>33.3 mM</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

**ATP stock**

Make stock at 53 mM (29.2 mg/ml) in H₂O. Make aliquots of 140 ul each and store @-20C. One aliquot is used for 10 ml of reaction buffer (final conc. of ATP being 750 uM)

**Luciferin stock**

Make stock at 17.6 mM (5.2 mg/ml). Make aliquots of 450 ul, which is the volume needed for 10 ml of reaction buffer (final conc. of luciferin being 800 uM). Keep aliquots @-20C.

Mix all 3 components (buffer, ATP and luciferine stock) and add water to 10 ml.