**LM031-2 – TGFb/Alda-1 treatment condition test**

The purpose of this test is to find the approximate treatment conditions which allow Alda-1 to attenuate TGFb response in IMR90 cells. This is a follow-up to LM031-1, in which I tried 36 different conditions at low resolution. Here, I will pursue the most promising conditions in greater detail.

I will plate four copies of the below design. Two will be for RNA and two for protein. Of each pair, one will be pretreated with Alda-1 before TGFb and one will be treated concurrently.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** |
| **A** | unstim DMSO | unstim DMSO | stim DMSO | stim DMSO |
| **B** |  |  | stim Alda20 | stim Alda20 |
| **C** |  |  | stim Alda40 | stim Alda40 |

Day 1: cell plating

1. Plate 150k IMR90 cells/well.
   1. R/S 4.95M cells in 33mL media and pipet 1 mL into each well. Top with another mL of media afterward.

Day 2: low sera and Alda-1 pretreatment

1. Make **0.25% FBS** media mixtures for pretx wells:
   1. 20uM Alda-1: 9 mL media + 18uL 10mM Alda-1
   2. 40uM Alda-1: 9 mL media + 36uL 10mM Alda-1
   3. DMSO: 9 mL media + 36uL DMSO
2. Pipet 1mL respective media onto each well.
3. Pipet 0.25% FBS

Day 3: TGFb and Alda-1 concurrent treatment

Will need 51mL 0.25% FBS media, 8.75uL TGFb, 64uL DMSO, and 88uL 10mM Alda-1.

1. Make +TGFb media:
   1. 35mL 0.25% FBS media + 8.75uL TGFb (4ng/uL)
2. Make +Alda-1 media:
   1. 20uM Alda-1 +TGFb: 9mL +TGFb media + 18uL 10mM Alda-1
   2. 40uM Alda-1 +TGFb: 9mL +TGFb media + 36uL 10mM Alda-1
   3. TGFb +DMSO: 9mL +TGFb media + 36uL DMSO
   4. DMSO: 9mL 0.25% FBS media + 36uL DMSO
3. Pipet 1mL respective media onto each well.
4. Incubate O/N.

Day 5: harvest

RNA

1. Wash wells 2x with ice cold PBS.
2. Aspirate excess PBS.
3. Pipet 600uL RLT +DTT onto each well.
4. Transfer RLT +DTT into 2mL round bottom tubes.
5. Isolate RNA on Qiacube.
6. Record RNA quality information from Nanodrop below.

Protein

1. Wash 2x with ice-cold PBS.
2. Aspirate remaining PBS off wells.
3. Pipet 75uL RIPA +PI onto each well.
4. Scrape wells and transfer to 1.5mL tubes.
5. Centrifuge 10min 4C 14,000 x g.
6. Transfer supernatant to a new tube and vortex.
7. Place 10uL supernatant + 40uL PBS in a 0.5mL tube.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| N**ame** | **Number on tube** | **Concentration (ng/uL)** | **260/280** | **260/230** |
| unstim DMSO pretx rep1 | 1 |  |  |  |
| unstim DMSO pretx rep2 | 2 |  |  |  |
| TGFB DMSO pretx rep1 | 3 |  |  |  |
| TGFB DMSO pretx rep2 | 4 |  |  |  |
| TGFB Alda20 pretx rep1 | 5 |  |  |  |
| TGFB Alda20 pretx rep2 | 6 |  |  |  |
| TGFB Alda40 pretx rep1 | 7 |  |  |  |
| TGFB Alda40 pretx rep2 | 8 |  |  |  |
| unstim DMSO conc rep1 | 9 |  |  |  |
| unstim DMSO conc rep2 | 10 |  |  |  |
| TGFB DMSO conc rep1 | 11 |  |  |  |
| TGFB DMSO conc rep2 | 12 |  |  |  |
| TGFB Alda20 conc rep1 | 13 |  |  |  |
| TGFB Alda20 conc rep2 | 14 |  |  |  |
| TGFB Alda40 conc rep1 | 15 |  |  |  |
| TGFB Alda40 conc rep2 | 16 |  |  |  |