**LM031-1 – TGFb/Alda-1 treatment condition test (coarse focus)**

The purpose of this test is to find the approximate treatment conditions which allow Alda-1 to attenuate TGFb response in IMR90 cells.

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| --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **Plate 1: 24h** |
| **A** | TGFb +10uM Alda-1 pretx | TGFb +10uM Alda-1 | TGFb +DMSO pretx | TGFb +DMSO |  |
| **B** | TGFb +20uM Alda-1 pretx | TGFb +20uM Alda-1 | Alda-1 40uM pretx | Alda-1 40uM |  |
| **C** | TGFb +40uM Alda-1 pretx | TGFb +40uM Alda-1 | DMSO pretx | DMSO |  |
|  |  |  |  |  |  |
|  | **1** | **2** | **3** | **4** | **Plate 2: 48h** |
| **A** | TGFb +10uM Alda-1 pretx | TGFb +10uM Alda-1 | TGFb +DMSO pretx | TGFb +DMSO |  |
| **B** | TGFb +20uM Alda-1 pretx | TGFb +20uM Alda-1 | Alda-1 40uM pretx | Alda-1 40uM |  |
| **C** | TGFb +40uM Alda-1 pretx | TGFb +40uM Alda-1 | DMSO pretx | DMSO |  |
|  |  |  |  |  |  |
|  | **1** | **2** | **3** | **4** | **Plate 3: 72h** |
| **A** | TGFb +10uM Alda-1 pretx | TGFb +10uM Alda-1 | TGFb +DMSO pretx | TGFb +DMSO |  |
| **B** | TGFb +20uM Alda-1 pretx | TGFb +20uM Alda-1 | Alda-1 40uM pretx | Alda-1 40uM |  |
| **C** | TGFb +40uM Alda-1 pretx | TGFb +40uM Alda-1 | DMSO pretx | DMSO |  |

Day 1: cell plating

1. Plate 200k IMR90 cells/well.
   1. R/S 7.6M cells in 38mL media and pipet 1 mL into each well.

Day 2: low sera and Alda-1 pretreatment

1. Make **0.25% FBS** media mixtures for pretx wells:
   1. 10uM Alda-1: 4mL media + 4uL 10mM Alda-1
   2. 20uM Alda-1: 4 mL media + 8uL 10mM Alda-1
   3. 40uM Alda-1: 8 mL media + 32uL 10mM Alda-1
   4. DMSO: 8 mL media + 32uL DMSO
2. Pipet 1mL respective media onto each well.

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. 10uM Alda-1 +TGFb: 8mL +TGFb media + 8uL 10mM Alda-1
   2. 20uM Alda-1 +TGFb: 8mL +TGFb media + 16uL 10mM Alda-1
   3. 40uM Alda-1 +TGFb: 8mL +TGFb media + 32uL 10mM Alda-1
   4. 40uM Alda-1: 8mL 0.25% FBS media + 32uL 10mM Alda-1
   5. TGFb +DMSO: 8mL +TGFb media + 32uL DMSO
   6. DMSO: 8mL 0.25% FBS media + 32uL DMSO
3. Prepare mentally/emotionally/spiritually for step 4.
4. Pipet 1mL respective media onto each well. Don’t screw up.
5. Incubate O/N.
6. High five self for successful heist.

Days 4-6: harvests

1. Remove plate to be harvested that day.
2. Wash wells 2x with ice cold PBS.
3. Aspirate excess PBS.
4. Pipet 600uL RLT +DTT onto each well.
5. Transfer RLT +DTT into 2mL round bottom tubes.
6. Isolate RNA on Qiacube.
7. Record RNA quality information from Nanodrop (next page).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Number on tube** | **Concentration (ng/uL)** | **260/280** | **260/230** |
| 24h TGFb Alda10 pretx | 1 |  |  |  |
| 24h TGFb Alda20 pretx | 2 |  |  |  |
| 24h TGFb Alda40 pretx | 3 |  |  |  |
| 24h TGFb Alda10 conc | 4 |  |  |  |
| 24h TGFb Alda20 conc | 5 |  |  |  |
| 24h TGFb Alda40 conc | 6 |  |  |  |
| 24h TGFb DMSO pretx | 7 |  |  |  |
| 24h unstim Alda40 pretx | 8 |  |  |  |
| 24h unstim DMSO pretx | 9 |  |  |  |
| 24h TGFb DMSO conc | 10 |  |  |  |
| 24h unstim Alda40 conc | 11 |  |  |  |
| 24h unstim DMSO conc | 12 |  |  |  |
| 48h TGFb Alda10 pretx | 13 |  |  |  |
| 48h TGFb Alda20 pretx | 14 |  |  |  |
| 48h TGFb Alda40 pretx | 15 |  |  |  |
| 48h TGFb Alda10 conc | 16 |  |  |  |
| 48h TGFb Alda20 conc | 17 |  |  |  |
| 48h TGFb Alda40 conc | 18 |  |  |  |
| 48h TGFb DMSO pretx | 19 |  |  |  |
| 48h unstim Alda40 pretx | 20 |  |  |  |
| 48h unstim DMSO pretx | 21 |  |  |  |
| 48h TGFb DMSO conc | 22 |  |  |  |
| 48h unstim Alda40 conc | 23 |  |  |  |
| 48h unstim DMSO conc | 24 |  |  |  |
| 72h TGFb Alda10 pretx | 25 |  |  |  |
| 72h TGFb Alda20 pretx | 26 |  |  |  |
| 72h TGFb Alda40 pretx | 27 |  |  |  |
| 72h TGFb Alda10 conc | 28 |  |  |  |
| 72h TGFb Alda20 conc | 29 |  |  |  |
| 72h TGFb Alda40 conc | 30 |  |  |  |
| 72h TGFb DMSO pretx | 31 |  |  |  |
| 72h unstim Alda40 pretx | 32 |  |  |  |
| 72h unstim DMSO pretx | 33 |  |  |  |
| 72h TGFb DMSO conc | 34 |  |  |  |
| 72h unstim Alda40 conc | 35 |  |  |  |
| 72h unstim DMSO conc | 36 |  |  |  |