**LM044-4: Alda-1/daidzin concentration test in bleo senescence induction with SA-B-Gal readout**

**Repeated this experiment because I used crazy high dmso concentrations in LM044-3 which obviously killed the cells. Keeping it under 0.5% this time like I was supposed to in the first place**

**Day 0-6: plate cells, treat, incubate, harvest**

1. A549 cells were trypsinized and plated 20k cells/well in 24 wells of a 24-well plate
2. Cells were incubated overnight to adhere.
3. Prepare 10 3mL aliquots of media and add compounds:

|  |  |  |
| --- | --- | --- |
| **Alda-1** |  |  |
| **conc uM** | **[stock] mM** | **vol stock uL** |
| 0 | 0 | 1.2 |
| 5 | 10 | 1.5 |
| 10 | 50 | 0.6 |
| 20 | 50 | 1.2 |
| 40 | 100 | 1.2 |
|  |  |  |
| **Daidzin** |  |  |
| **conc uM** | **[stock] mM** | **vol stock uL** |
| 0 | 0 | 1.44 |
| 5 | 100 | 0.9 |
| 10 | 300 | 0.6 |
| 20 | 500 | 0.72 |
| 40 | 500 | 1.44 |

1. Pipet 1mL media onto respective wells:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Alda-1 veh | Alda-1 5uM | Alda-1 10uM | Alda-1 20uM | Alda-1 40uM | untx | vehicle |
| **Alda-1 veh** | **Alda-1 5uM** | **Alda-1 10uM** | **Alda-1 20uM** | **Alda-1 40uM** | **untx** | **bleo** |
| daidzin veh | daidzin 30uM | daidzin 60uM | daidzin 120uM | daidzin 240uM | untx | vehicle |
| **daidzin veh** | **daidzin 30uM** | **daidzin 60uM** | **daidzin 120uM** | **daidzin 240uM** | **untx** | **bleo** |

1. Spike 16.66uL 3mg/mL bleo into each +bleo well and 16.66uL ultrapure H2O into vehicle wells.
2. At treatment day 3, aspirate media and replace with the same formulations applied on treatment day 1.

**Day 6: SA-B-Gal harvest**

1. Dilute the 10X fixative solution with water.
   1. Make 25mL: 2.5mL 10x + 22.5mL H2O
2. Remove media from cells on 3cm plates.
3. Wash wells/plates with PBS.
4. Add 1mL 1x Fixative Solution to wells/plates.
5. Fix 10-15min at RT.
6. Rinse wells/plates 2x with 1X PBS. Leave at 4C until stain is performed.

**SA-B-Gal staining and WB**

**Solution preparation**

1. Resuspend the 10x staining solution by heating to 37C with agitation. Dilute the solution to 1x with H2O. Make 12.09mL: 1.209mL 10x + 10.881mL H2O
2. Dissolve 20mg X-gal in DMSO to make 20mg/mL stock. Store excess at -20C for 1 month. **Must prepare in polypropylene plastic or glass.**
3. B-gal staining solution: For each well to be stained, prepare the following:
   1. 930uL 1x staining solution
   2. 10uL 100x Solution A
   3. 10uL 100x Solution B
   4. 50uL 20mg/mL X-gal stock solution
   5. **pH the final solution. pH should be 5.9-6.1. DO NOT SKIP THIS!!**

**Staining procedure**

1. Aspirate PBS from each plate/well.
2. Add 0.5mL B-gal staining solution to each well. Seal plate with parafilm.
3. Incubate 37C at least O/N in dry incubator.
4. Check blue staining under a microscope.
5. For long-term storage, remove the staining solution and overlay with 70% glycerol. Sore at 4C.

.