**LM044-3: Alda-1/daidzin concentration test in bleo senescence induction with SA-B-Gal readout**

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

1. A549 cells were trypsinized and plated 30k cells/well in 24 wells of a 24-well plate
2. Cells were incubated overnight to adhere.
3. Wash cells 2x PBS. Treat with 50ug/mL bleomycin.
   1. [stock bleo] = 3mg/mL
      1. 12.8mL media +216.67uL bleo
      2. 12.8mL media + 216.67uL ultrapure H2O
   2. Divide media into 12 2mL aliquots and add compounds:

|  |  |  |
| --- | --- | --- |
| **Tube** | **Compound** | **vol compound (uL)** |
| 0uM | DMSO | 16 |
| 5uM | Alda-1 | 1 |
| 10uM | Alda-1 | 2 |
| 20uM | Alda-1 | 4 |
| 40uM | Alda-1 | 8 |
| 80uM | Alda-1 | 16 |
| 0uM | DMSO | 80 |
| 40uM | daidzin | 8 |
| 60uM | daidzin | 12 |
| 100uM | daidzin | 20 |
| 200uM | daidzin | 40 |
| 400uM | daidzin | 80 |

1. Apply media to plate as follows:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **0uM Alda-1** | **5uM Alda-1** | **10uM Alda-1** | **20uM Alda-1** | **40uM Alda-1** | **80uM Alda-1** | **bleo** |
| 0uM Alda-1 | 5uM Alda-1 | 10uM Alda-1 | 20uM Alda-1 | 40uM Alda-1 | 80uM Alda-1 | mock |
| **0uM daidzin** | **40uM daidzin** | **60uM daidzin** | **100uM daidzin** | **200uM daidzin** | **400uM daidzin** | **bleo** |
| 0uM daidzin | 40uM daidzin | 60uM daidzin | 100uM daidzin | 200uM daidzin | 400uM daidzin | mock |

1. Incubate cells 48h.
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 17mL: 1.7mL 10x + 15.3mL H2O
2. Remove media from cells on 3cm plates.
3. Wash wells/plates with PBS.
4. Add 3mL 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 15.81mL: 1.581mL 10x + 14.229mL 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 1mL 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.

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