**Titrate MG**

1. Use a 6 well plate, 2ml of medium, 0,5x106cells (allow to grow and attach overnight). Usually 3 picomoles of human cells is equal to 106 cells.

**Treatments**

* **Cell harvest and cell media of treatments** time (min) after MG incubation: 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60.
* **Media alone without cells** 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60.

**Total wells** 36 (MG treatments) + 3 (control treatments time 60 min w/o MG)

**Media sample** 36 (Media cell treatments) + 33 (media alone)

**Media**

Made 160ml media + MG (Stock 150mM use 27ul for 25uM concentration).

Made 100ml media alone

**Cells**

25x106 cells

4 flask T75 of cells

1. Use 25uM MG + Media.
2. Save 40ul of media in an eppi.
3. Wash the cells with 5mM **HEPES?.**
4. Put the 6 well plate in a container of liquid nitrogen, the container should have around 1,5-2cm of liquid nitrogen, enough to freeze the bottom of the plate without getting inside the plate.
5. Keep the plate in the -20C for short periods of time, in the -80C for longer ones.