

## RNAi SCREENING IN DROSOPHILA CELLS

### Equipment/material needed:

1. Multidrop (ThermoElectron Corporation, #5840300)
2. Mithras Platereader LB940 (Berthold)
3. 384well LIA white plates (Greiner, #781073)
4. Alufoil seals (Costar, #6569)
5. DDAB (Sigma, D2779)
6. Schneider's Drosophila Medium (Invitrogen, #11720-034)
7. Celltiter Glow (Promega, #G7571)
8. VP186L (24-channel wand) (VP Scientific, #VP186L)

**Assay:** Viability assay  
**Plate type:** LIA plate white (Greiner, #), 384 well  
**Cell type:** Drosophila S2  
**dsRNA/well:** 5 $\mu$ l of 50ng/ $\mu$ l (total per well: 250ng)

### Preparing the plates

- Thaw the assay plates for approximately 1 hour at room temperature
- Spin down the plates for 1min at 1000rpm
- Wipe each plate with 70% ethanol (to avoid contaminations during stacking)
- Remove the lids of the plates
- Pipette the controls in the corresponding wells (5 $\mu$ l of 50ng/ $\mu$ l dsRNA)
- Cover the plate on top of each stack with a lid (again to avoid contaminations)

*Day 1:*

### Prepare the multidrop

- Rinse the tube with:
  - 70% Ethanol
  - ddH<sub>2</sub>O
  - serum free Schneider's Drosophila Medium (Invitrogen)

### Preparation of the cell suspension

- Scrape the cells of each flask, pool and count them
- To set up the desired cell count (for example 2 mio cells per ml) spin down the required volume of cell suspension. *Note: to account for the dead volume of the multidrop an additional of 8ml is required.*

**Distribution:** Internal and External OK

- Remove the supernatant and resuspend the cell pellet in the calculated volume of serum-free medium (SFM)
- Now your cells are ready for screening! Immediately process with the next steps because your cells start starving in SFM...

### **Performing the assay**

- Seed 15µl of your prepared cell suspension (in this example: 30.000 cells/well) with the Multidrop. *Note: adjust cell number depending on cell type and duration of assay*
- Starve the cells for 45-60min at room temperature
- Add 20µl serum containing Schneider's Drosophila and DDAB (stock: 0.4mg/ml, use 0.2µl per well, depending on the cell line this might differ) medium on top of the cells. *Note: DDAB is a cationic liposome reagent to improve the transfection of dsRNA into the cell.*
- Seal the plates with alufoil and spin them 1min at 1000rpm
- Incubate the plates at 25°C

*Day 2-4:*

- Incubation at 25°C

*Day 5:*

### **Readout**

- Spin the plates 1min at 1000rpm
- Remove the seals
- Remove the medium from wells using a 24 channel wand
- Add 20µl Celltiter Glow per well (1:3 diluted in PBS)
- Incubate 15min at RT (protect plates from light!)
- Start readout using the Mithras plate reader (no filter)