Quantitative Fluorescence Measurement Using Modulus Fluorometer

By Chris Song, 1-29-06; Commented by TCH

1. Add 4 volumes of water to 1 volume of **5X Cell Lysis Buffer** (stored at -20°C, Promega). Note: Cell Culture Lysis Reagent provides efficient lysis within minutes (5min) (From Promega, as same as the lysis buffer used in Luciferase assay).

2. Aspirate the medium of every well carefully and quickly. Add enough 1X lysis buffer to cover the cells. **Usually, add 150ul lysis buffer per well of 24 wells plate**. Rock culture dishes several times to ensure complete coverage of the cells with lysis buffer. **Note:** It can save time to use tips to disturb the buffer and cells. You should also lyse some wells with cells only (i.e., no treatment) as **true background control**.

3. Collect the cell lysate and transfer to 1.7ml microfuge tubes, centrifuge at top speed for 1 min at room temperature.

4. Change the optical kit to **Blue kit** (multifunctional Module luminometer for fluorescence, luminescence, and absorbance from Turner Biosystems .Ltd):
   - Power the Modulus **OFF**
   - Grasp the handle of the other Optical Kit and pull it out of the module. (Normally we use it for luciferase assay)
   - Grasp the handle of the Optical Kit and align the kit with the sample compartment
   - Press down firmly to lock the Optical Kit in place
   - Close the lid and power **ON** the Modulus. Use the touch screen to identify the type of Optical Kit installed

   Note: Allow the Modulus a 5-minute warm up period before calibration and measurement.

5. Transfer at least 100ul lysate into cuvettes (i.e., small tubes) (100 micrlTR, Turner. Ltd).

6. Open the lid of the Modulus and insert the cuvettes. Close the lid. Touch "***Measure Fluorescence***" to commence measurement. The Modulus will measure the sample for 6 seconds. Read down the reading.