

Reporter Assay Using Dual-Glo

1. If using the Chameleon Plate reader, prior to reading the plates, **place a sticker on the bottom of the plate** (stickers are kept next to plates). The Chameleon reads from above the plate so the luminescence will only shine upwards if the sticker is placed. Other plate readers read from the bottom. If not using the Chameleon, will need to check the manual of the plate reader to determine if covering the bottom of the plate is necessary.
2. To read the transfection, **remove media and add 20 μ l of PBS.**
3. **Add 20 μ l of Dual-Glo reagent.** (When thawed for the first time, aliquot 5.5ml aliquots into 15ml conical tubes. Thaw aliquot @ RT for use; mark the aliquot if you refreeze).
 - a. **Incubate 10'** then **read** on plate reader using the "Lumin 5sec TCL" parameter file. (May incubate for at least 1 hr).
 - b. **IF READINGS ARE ABOVE 100,000 RLU, WAIT ONE-TWO HOURS BEFORE ADDING THE STOP-GLO SO THAT THE FIREFLY DOES NOT BLEEDOVER TO THE RENILLA READING!!!!**
4. **Add 20 μ l of Stop-Glo reagent.** (Dilute substrate 1:100 in dilution buffer; substrate is @ -20°C; dilution buffer is at 4°C).
 - a. NEVER leave Stop-Glo reagent UNCAPPED OR AT ROOM TEMP. It is highly volatile and will evaporate. Aliquot amount of stop-glo diluent you will need then bring to freezer and uncap substrate tube only long enough to remove desired amount (this is very important or we will run out of substrate long before diluent).
 - b. **Incubate 10'. Read** on plate reader using the "Lumin 5sec TCL" parameter file. (May read for at least 1 hr)

NOTES:

1. If using a 12-well plate and needed to lyse cells and scrap, Promega does not suggest using lysis buffers with the Dual-Glo system. Instead, they suggest scraping the cells in PBS, pelleting them, and transferring the entire pellet in a 20 μ l volume of PBS to a non-sterile 96w plate. Then proceed from step 4. Though there is a little more processing, there has been great success for experiments that require myotube formation using this alteration.
2. Note that the "Lumin 5sec TCL" parameter file is a "direct" mode file. This is equivalent to HIGH SENSITIVITY! If sensitivity is too high, you can use the "Lumin filtered" parameter file (filter mode). As an aside, the filter mode further reduces cross-talk between wells.
3. See "Chameleon Plate reader" Protocol for more detail on using the instrument

ITEM	Catalog number	Vendor
Dual-Glo TM Luciferase Assay System	PRE2980 (10x100ml)	Fisher
White stickers for bottom of 96w-plate	6005199	Perkin Elmer
96 well white plates, sterile, clear bottom	07-200-566	Fisher (Costar #3610)
96 well solid white plate, nonsterile	07-200-589	Fisher (Costar #3912)