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NBP2-25287 Protocol

Product Handling Protocol (NBP2-25287)

Note: Luciferase assays may be conducted immediately or the plates may be frozen at -80C (freezing generally increases cell lysis and luciferase signal). If using frozen plates, thaw and bring to room temperature before assaying.

1) Reconstitute 100X Substrate.
Add 1mL Substrate Solvent to tube of lyophilized Assay Substrate. Dissolve completely. Protect from light and minimize time at room temperature. 100x Substrate may be stored at -20C and protected from light for 2-3 weeks. For best results, use freshly reconstituted substrate.

2) Prepare Assay Solution (for 1000 tests).
Thaw 100mL bottle of Assay Buffer in room temperature water bath and add 1mL of reconstituted 100x Substrate just prior to use. Prepare Assay Solution (buffer + substrate mix) fresh for each use and use within 2-3 hours. To assay fewer wells, make up only what you need and store remaining substrate and buffer separately at -20C. For best results, avoid additional freeze-thaw cycles. To thaw re-frozen buffer, incubate in a warm (37C) water bath for at least 1 hour and mix well to ensure that all components go back into solution.

3) Add Assay Solution to wells.
Use a multi-channel pipettor to add 100uL Assay Solution (buffer + substrate) directly to each sample well (100uL cells + media) in a white 96-well plate. The Assay Buffer also serves as a lysis buffer. If cells were grown in another plate or flask, transfer samples to a white 96-well plate in 100uL total volume (media or PBS).

4) Incubate for 30 minutes at room temperature.
Cover plate and protect from light. If assaying more than one plate, stagger addition of Assay Solution so that each plate incubates for 30 minutes before reading.

5) Read each well for 2 seconds in a plate luminometer.
A recommended luminometer is the SpectraMax L (Molecular Devices)