Preparation of Luciferin for *In Vitro* and *In Vivo* Bioluminescent Assays

Preparation of Luciferin for *In Vitro* Bioluminescent Assays

Materials

- D-Luciferin Firefly, potassium salt, 1.0 g /vial (PerkinElmer Part Number #122799)
- Sterile water
- Complete media

Cells should be seeded in a plate overnight or several hours prior to assay to allow the cells to attach to the bottom. Suspension cell lines can be seeded in the working solution in the plate for direct incubation and imaging.
Doubling time should be considered for cell counting if doubling time is relatively short.

Procedure

1. Prepare a 200X Luciferin stock solution (30 mg/ml) in sterile water. ***Note:*** *One can either reconstitute the entire 1.0 g of D-Luciferin in 33.3 mL of sterile water to make the 30 mg/mL (200x) stock solution, or reconstitute the quantity of D-Luciferin necessary for an individual experiment.*

2. Mix gently by inversion until Luciferin is completely dissolved. *****

3. Prepare a 150 µg/ml working solution of D-Luciferin in pre-warmed tissue culture medium.
   Quick thaw 200X stock solution of Luciferin and dilute 1:200 in complete media (150 µg/ml final concentration).

4. Aspirate media from cultured cells.

5. Add 1x Luciferin solution to cells just prior to imaging. ***Note:*** *Incubating the cells for a short time at 37 °C before imaging can increase the signal. Incubation time is dependent on the specific cell type. Generally, a 10 min incubation is sufficient; test and adjust as needed.*

6. Check the *in vitro* bioluminescence using the IVIS imaging system every 10 min, up to 40 min, to determine the kinetic curve and find the peak imaging time point for each cell type.

Preparation of Luciferin for *In Vivo* Bioluminescent Assays

Materials

- D-Luciferin, Firefly, potassium salt, 1.0 g/vial (PerkinElmer Part Number #122799)
- DPBS, w/o Mg2+ and Ca2+
- Syringe filter, 0.2 um

Procedure

1. Prepare a fresh stock solution of Luciferin at 15mg/ml in DPBS. *
2. Filter sterilize through a 0.2 um filter.
3. Determine injection amount at 10 µL/g of body weight. Each mouse should receive 150 mg Luciferin/kg body weight. (e.g. For a 10 g mouse, inject 100 µL to deliver 1.5 mg of Luciferin.)
4. Inject the Luciferin intra-peritoneally (i.p.) 10-15 minutes before *in vivo* imaging, or as determined by kinetic curve. **

* *Immediate use of working solution after dilution is strongly recommended. If necessary, dissolved luciferin may be stored at 4°C for up to 3 weeks; however prolonged storage at either 4°C or -20°C may result in degradation of signal.*

** *A Luciferin kinetic curve should be performed for each new animal model to determine peak signal time. Please see our 'Determining the Luciferin Kinetic Curve for Your Model' instruction sheet available for download on our website.*

NOTE: Luciferin is a light sensitive reagent, and should be kept out of direct light as much as possible. We recommend that the luciferin be protected from light (e.g. covered with a light blocking material such as tin foil) during the entire assay, from the stock solution preparation until completion of the procedure.

For research use only. Not for use in diagnostic procedures.

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