Preparation of IVISbrite D-Luciferin for *in vitro* and *in vivo* bioluminescent assays

Preparation or IVISbrite™ D-luciferin for *in Vitro* Bioluminescent Assays

Materials

- IVISbrite D-Luciferin Potassium Salt, 1.0 g /vial (Revvity Part Number #122799)
- 1 x Phosphate Buffered Saline (PBS)
- 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 D-Luciferin stock solution (30 mg/ml) in 1xPBS.
2. Mix gently by inversion until D-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 D-Luciferin and dilute 1:200 in complete media (150 µg/ml final concentration).

4. Aspirate media from cultured cells.
5. Add 1x D-Luciferin solution to well plates just prior to imaging. Perform imaging immediately. If imaging cannot be performed immediately after adding D-Luciferin, keep plates on ice and avoid light.
6. Check the *in vitro* bioluminescence using the IVIS™ imaging system every minute, up to 20 minutes, to determine the kinetic curve and find the peak imaging time point for each cell type.

Preparation of D-Luciferin for *in vivo* bioluminescent assays

Materials

- IVISbrite D-Luciferin Potassium Salt, 1.0 g /vial (Revvity Part Number #122799)
- 1xPBS, w/o Mg^{2+} and Ca^{2+}
- Syringe filter, 0.2 µm

Procedure

1. Prepare a fresh solution of D-Luciferin at 30 mg/mL in 1xPBS*.
2. Filter sterilize through a 0.2 µm filter.
3. Calculate injection volume based on mouse bodyweight. (e.g. Dose at an injection volume of 5 mL/kg. For a 20g mouse, inject 100uL to deliver 150 mg/kg of D-Luciferin solution. For rat models, we recommend a dose volume of 10 mL/kg.)
4. Inject IVISbrite D-luciferin intra-peritoneally (i.p.) or subcutaneously (s.c) 10-15 minutes before *in vivo* imaging.

*Always use freshly prepared D-Luciferin prior to each imaging session. If necessary, IVISbrite D-luciferin solutions may me aliquoted and frozen at -20 °C for 6 months.

Note for both *in vitro* and *in vivo* assays: D-Luciferin is a light sensitive reagent, and should be kept out of direct light as much as possible. We recommend that the D-Luciferin be protected from light (e.g. covered with a light blocking material such as tinfoil) while in use.

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