

D-infraluciferin methyl ester user manual

1. Formulation of stock solution for *in vitro* and *in vivo* imaging (includes filter sterilisation step*(e))

- a. Carefully open the screw top 2 ml tube containing *D*-infraluciferin methyl ester.
- b. Slowly add 2 ml of sterile phosphate buffered saline (PBS) solution.
- c. Screw the tube lid back on securely.
- d. Gently invert the tube 4 times to solubilise. The ester forms an emulsion in PBS and this is normal.
- e. Next, filter sterilise using a 0.22 µ filter (recommended for *in vitro* work, necessary for *in vivo* work)*.
- f. The product is now ready for use as a stock solution at a concentration of 20 mg/ml or 62.425 mM. It should be used or kept in the dark (or protected from light) and on ice (or at 4°C) if use is delayed (by hours). It can then be allowed to come to room temperature and gently inverted 3 times to mix before use.

Alternatively, for longer term in vitro studies, a smaller amount of D-infraluciferin methyl ester may be weighed out and made into stock solutions of smaller volume. The remaining solid in the Bioflares tube (with cap screwed on) may be stored in the dark at -20°C (see Storage, below).

2. In vitro imaging in cells

- a1. Remove cell culture medium and add 1 mM *D*-infraluciferin methyl ester on to cells in PBS.
- a2. **Alternatively**, add stock solution of *D*-infraluciferin methyl ester directly to the culture medium to a final concentration of 1 mM. Mix well and wait 2-5 min before imaging.

Calculation for dilution to 1 mM:

Stock solution of D-infraluciferin methyl ester as prepared above is 62.425 mM. Therefore, dilute as follows:

$$1 / 62.425 \times \text{volume of culture medium } (\mu\text{l}) = \text{volume of stock solution to add } (\mu\text{l})$$

For example, in a single 2 ml (2000 µl) well of a 6-well tissue culture plate:

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$$1 / 62.425 \times 2000 \mu\text{l} = 32 \mu\text{l}$$

Therefore, you would need to add 32 μl of stock solution to each well required. If you require every well of the 6-well plate, you require a total of 192 μl of D-infraluciferin methyl ester stock solution. This is about 1/10 of the stock solution prepared from a single Bioflares 40mg aliquot.

An alternative is to prepare 1 mM solutions in PBS and remove the culture medium (if appropriate). This can half the cost, by imaging with 1 ml solution added in place of 2 ml culture medium, for example. You can use 0.5 ml for 12 well plates and 0.25 ml solution per well for 24 well plates and scale volumes in smaller plates accordingly. Use of a multi-well pipette is recommended where the 1mM solutions are dispensed from a sterile trough.

3. Imaging in mice: *in vivo* bioluminescence imaging

a. Filter (0.22 μm) sterilised 20mg/ml stock solution should be given to mice in a dose of 4mg per mouse by the intraperitoneal route¹.

This is 200 μl of stock solution per mouse. If there is a small loss from filtering, divide the solution from one aliquot equally between 10 mice, and calculate the dose by accounting for the volume change. One aliquot will image 10 mice effectively.

¹As with natural luciferin, infraluciferin methyl ester can be administered by many routes (e.g. intravenous). Dosages for other routes and different models may vary and it may be more applicable and cost-effective to use 2mg per mouse in some cases. This should be assessed in individual cases in terms of signal, e.g. light output from brighter *ilucs* may saturate at lower concentrations of infraluciferin due to improved enzyme properties.

For other models and organisms, please refer to the literature on dosing with natural luciferin to gauge how to range dosing with infraluciferin based on this manual. The normal dose of natural luciferin for a mouse is 2mg.

4. Storage in solution and longer term storage as solid

a. For optimal results avoid keeping D-infraluciferin methyl ester in stock solution for more than a day on ice or at 4°C (light protected) before use *in vitro* or *in vivo*, or freezing (see below). Freezing and re-using lower concentrations, such as 1mM solutions, should be avoided.

b. Freezing at -20°C (protected from light) sustains the life of stock solution by months but some activity reduction may be observed after such time.

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d. Tubes of *D*-infraluciferin methyl ester solid (not solution) can be stored in a -20°C freezer for ~2 years.
Some activity reduction may be observed after such time.