APPLICATION BRIEF

In Vivo Preclinical Imaging

PROTOCOL: *In Vivo* Imaging Protocol with RediJect D-Luciferin Ultra

XenoLight RediJect D-Luciferin Ultra

Part Number	770505
Properties	Yellow Colored Solution (D-Luciferin Potassium Salt in PBS)
Concentration	10 Sterile Vials Each Containing 850 µl of 30 mg/ml D-Luciferin Ultra
Storage and Handling	Store at ≤ -20 °C. Repeated Freeze Thaw is Not Recommended.

RediJect D-Luciferin Ultra has the advantage of utilizing fluorescent signal to normalize the bioluminescent signal. In a typical experiment using D-Luciferin Ultra, it is recommended to first obtain the fluorescent signal quantification and determine the average fluorescence signal of the group. For this calculation, it is important to omit outliers.

- Just before your experiment, remove a vial from the kit and place it in a 37 °C water bath for five minutes. Vortex the tube for one minute and it is ready to use.
- For *in vivo* imaging studies, we recommend intraperitoneal (i.p.) injection of RediJect D-Luciferin Ultra at 150 mg/kg (150 µl/mouse injection*) using a 25 gauge needle, usually with 1 cc syringe.
- 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.**
- Once plateau is determined, allow D-Luciferin Ultra to distribute in animals under conditions consistent with those the animals were under during kinetic curve generation, i.e. under anesthesia and warmed to 37 °C.
- Place fully anesthetized animals in the IVIS imaging system. Before bioluminescence imaging, fluorescence imaging is performed by setting the exposure time to one second and selecting excitation filter 745 nm and emission filter 800 nm.
- For quantifying fluorescent images, it is important to place the region of interest (ROI) away from the abdominal region where the substrate is i.p. injected to get a better read out of the systemic distribution of the substrate. For dorsal images, the ROI is drawn around the scruff area (back of neck) for quantification of the reference fluorescence signal, while for ventral images, the ROI is drawn around the thoracic region. With the Living Image software, fluorescence signal is measured in efficiency units.

* *Calculations based on a 30 g mouse*

Using the following formula, the % change in fluorescence signal for each mouse is determined:

FLI Normalization Factor (%) = (FLI signal – average FLI signal)/ average FLI signal

Using this average fluorescence signal, determine the percent change of fluorescence signal from the average fluorescence signal, for each mouse. If a particular mouse has greater than a 30% decrease in Fluorescent signal from the average, that mouse needs to be reimaged.

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