

Instructions for CAT#3481 Water-soluble 6h-F-Coelenterazine (6h-F-CTZ-SOL) for *in vivo* use

Content:

500 µg 6h-F-CTZ per vial of sterile, water-soluble 6h-F-Coelenterazine for the *in vivo* use with NLuc Luciferase (NanoLuc, NanoKAZ, Oplophorus Luc)

Storage:

Please store unopened vials at -80°C, for shorter periods of time at -20°C. Keep dark and dry. Reconstituted Coelenterazine should be used within a day⁸; do not refreeze.

Usage:

- Carefully add 100 µL of sterile, distilled water to the top of the cake and pre-heat hotplate to 80°C.
- Re-cap the vial with the rubber stopper and allow the cake to mostly hydrate before heating. This can take some time (approx. 5-10 min). Mix by vortex at a high speed to knock any stuck pieces into the mixture if necessary.
- Place the vial on top of your hotplate. Let it sit there until the contents are fully dissolved (usually <1min). The liquid will be quite viscous, so it may be necessary to mix it by vortex to fully dissolve any material. **Monitor this step carefully** to prevent too much pressure building up in the vial.
- Once dissolved, remove the vial from the hotplate. The solution will be at a concentration of 5 mg/ml. To further dilute, add the substrate to a larger volume of sterile water, do not add water to the dissolved substrate. **Do not use PBS.**
- 100 µg of substrate (=20 µl) per 25 gram mouse is a good starting point for imaging purposes
- Depending on the amount of 6h-F-CTZ you want to inject use following amounts of **sterile water** (do not use PBS) to further dilute the substrate:

<u>Desired CTZ amount per injection</u>	<u>Total volume</u>	<u>volume per injection</u>
50 µg	500 µl	10 injections of 50 µl
100 µg	250 µl	5 injections of 50 µl
250 µg	200 µl	2 injections of 100 µl
500 µg	100 µl	one injection of 100 µl

Draw up the desired volume (see table above) with Insulin syringe (e.g. BD cat. # 328430). Inspect for and remove any air bubbles in the syringe and flush the needle. **Inject via tail vein to ensure optimal distribution throughout the body.** The advantage of using Insulin syringes is their very low (<2µl) holdup volume! **Inject slowly.**

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*Disclaimer: No adverse effects have been observed in mouse studies conducted to date. However, these findings do not guarantee the absence of side effects or unforeseen biological effects under all experimental conditions. This compound is provided for research use only, and users should exercise appropriate scientific judgment when designing and conducting experiments.