

Practical Notes for teLuc-DTZ and Antares2-DTZ (updated 07/29/2019)

There is considerable interest in adapting teLuc-DTZ or Antares2-DTZ for various biological and biomedical studies. The plasmids are now available from Addgene. You may purchase DTZ from Haoyuan Chemexpress Co., Ltd. (we do not make any profit by suggesting this company, but we have tested their DTZ compound). DTZ should be shipped in **nitrogen-filled-glass bottle**, or on dry ice if oxygen is not completely removed. For long-term storage, DTZ should be kept as solid in **-80°C freezers**.

To make a stock solution for DTZ, first, a premixture is prepared by dissolving 17.6 mg of L-ascorbic acid in 10 mL ethanol and 10 mL 1,2-propanediol; next, 1 mg of DTZ is dissolved in 88 μ L of the premix, resulting in a 30 mM DTZ stock solution containing 5 mM L-ascorbic acid. The stock solution should be stable for a few months in -80°C freezers. It may also be aliquoted to 10-20 μ L each at -80°C for the convenience of use. **We want to note that this new formulation greatly enhances substrate stability, compared to the conventional acidic alcohol solution.** To use teLuc/DTZ for protein-based *in vitro* assays and gain glow-type emission, please use the following assay buffer: 1 mM CDTA, 0.5% Tergitol NP-40, 0.05% Antifoam 204, 150 mM KCl, 100 mM MES pH 6.0, 1 mM DTT, and 35 mM thiourea. *Please also note that the amount of enzyme should be much lower than the substrate in order to gain extended emission.*

To use DTZ for animal studies, you may directly dissolve 1 mg DTZ in 375 μ L of 50% (w/v) hydroxypropyl- β -cyclodextrin (Sigma Cat# 389145) in H₂O to gain a final solution containing 7 mM DTZ. This ready-for-use solution should be stored at -80°C. During usage, you may keep the solution on ice for a few hours. Using this recipe, you can easily deliver ~ 0.3-1.4 μ mol DTZ into each ~ 20 g mouse via IV (or up to 3.5 μ mol/500 μ L via IP).

Although cautions have to be made to maintain the *in vitro* stability of DTZ, background bioluminescence is generally not an issue for *in vivo* applications of DTZ. Background bioluminescence from DTZ is negligible compared to real signals. We have examined this with untreated blank BALB/c and C57BL/6 mice, mice transfected with empty vectors, and mice injected with cells containing no luciferase.

For systematic delivery, we suggest you compare IP and IV injections of DTZ. Depending on different applications, either IP or IV may give better signals.

When exogenous cells expressing teLuc were injected into immunocompetent BALB/c mice, we observed fast clearance. teLuc in dead cells or released from dead cells is still enzymatically active, since teLuc is independent of ATP and highly resistant to denaturation and unfolding. For this particular case, you would observe diffused signals, which are not background but from enzymes in blood and urine. When comparing results with firefly luciferase or its derivatives, please note that firefly luciferase may only function in live cells where there are enough ATP.

Although DTZ has not yet been frequently used for animal imaging because our paper was only published several months ago, there are a plethora of literatures which used DTZ analogs, including CTZ and furimazine, for *in vivo* imaging. You may refer to these publications to better understand the potential of teLuc-DTZ and Antares2-DTZ for *in vivo* imaging. We select a few papers below:

Bhaumik S, Gambhir SS. *Optical imaging of Renilla luciferase reporter gene expression in living mice.* Proc Natl Acad Sci U S A. 2002, 99(1):377-82.

Loening AM, Wu AM, Gambhir SS. *Red-shifted Renilla reniformis luciferase variants for imaging in living subjects.* Nat Methods. 2007, 4(8):641-3.

Saito, K, Nagai T, et al., *Luminescent proteins for high-speed single-cell and whole-body imaging.* Nat Commun. 2012, 3: 1262.

Chu J, Lin MZ, et al., *A bright cyan-excitable orange fluorescent protein facilitates dual-emission microscopy and enhances bioluminescence imaging in vivo*. Nat Biotechnol. 2016, 34(7):760-7.

Franz X Schaub, Antonio L Amelio, et al., *Fluorophore-NanoLuc BRET reporters enable sensitive in vivo optical imaging and flow cytometry for monitoring tumorigenesis*. Cancer Res. 2015, 75(23):5023-33.

PS: Our lab has recently published two papers related to teLuc-DTZ and Antares2-DTZ. The *Biochemistry* paper has more information about the use of teLuc-DTZ and Antares2-DTZ in mice. The *ACS Chem Biol* paper reports new variants derived from teLuc and Antares2. These new variants are probably better suited for *in vivo* applications due to improved substrate water solubility and further red-shifted emission.

H.W. Yeh, T. Wu, M. Chen, and H-w. Ai*, "Identification of Factors Complicating Bioluminescence Imaging", *ACS Biochemistry*, 2019, DOI: 10.1021/acs.biochem.8b01303

H.W. Yeh, Y. Xiong, T. Wu, M. Chen, A. Ji, X. Li, and H-w. Ai*, "ATP-independent bioluminescent reporter variants to improve in vivo imaging", *ACS Chemical Biology*, 2019, DOI: 10.1021/acscchembio.9b00150