Quick Manual

Renilla GLOW-Juice BIG KIT (1 02 532)

Components include:

Renilla GLOW-Juice	**100 ml** Buffer for measuring Renilla-Luciferase – without substrate. **Store at +4°C.**
Coelenterazine(CTZ)	**1 vial lyophilisated substrate for 10 ml Renilla GLOW-Juice** (synth.) Coelenterazine for Renilla- and Gaussia- Luciferase **Store at -80°C in the dark**.
Reconstruction buffer	**2 ml** for dissolving the lyophilisated Coelenterazine **Store at -80°C in the dark**.
2x Lysis-Juice 2	**2 x 10ml dual concentrated** Lysis-Buffer without detergents, for measurement of marine luciferases (Renilla/Gaussia) in mammalian cells**. Store at +4°C.**

Reconstruction:

This Renilla GLOW-Juice BIG KIT includes 100 ml Test-Systems for Renilla-Luciferase. Note: If the lyophilisated Coelenterazine is dissolved in the Reconstruction buffer the stability will decrease after 30 Days.
Please pipette the 2 ml Recontruction buffer into the brown glass tube of lyophilisated CTZ. It result a **50 x stock solution** that will be stable for at least **30 days** after reconstruction **(store at -80°C!!!).** The calculated amount of Coelenterazine stock solution has to be mixed into the measuring Renilla GLOW-Juice **shortly before use** (2µl CTZ into 100 µl Renilla GLOW-Juice).
The reagents should reach at least room temperature (20-25°C) before starting measuring the luciferases!
Remainders of the mixed Renilla GLOW-Juice should not be frozen again because it will loose noticeable its activity.

Preparation of Cell Lysates:

Renilla GLOW-Juice BIG KIT includes Lysis-Juice 2. This buffer doesn't contain detergence, is dual concentrated and suitable for mammalian cells which were transfected with **Renilla/Gaussia/Firefly Luciferase.** Please dilute the dual concentrated lysis buffer with water or within your cell culture!

Standard protocol for Cells cultured in multiwell-plates

Required final volume of Lysis-Juice 2 per well:

Culture Plate	Vol. Lysis-Juice
6-well	500µl
12-well	250µl
24-well	100µl
48-well	65µl
96-well	20µl

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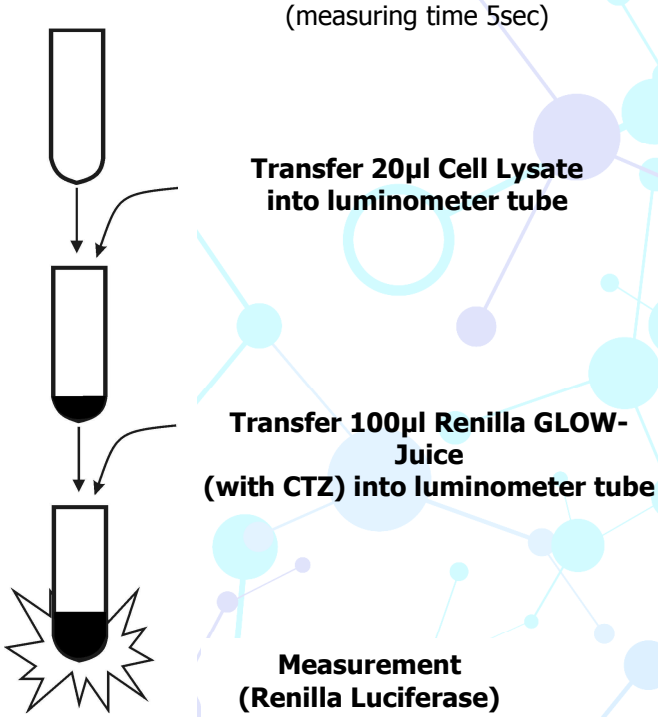
- Remove the growth medium from your adherent cells and wash the monolayer two or three times with phosphate buffered saline (PBS)
- Add the required volume of Lysis-Juice to each well (see Table.1)
- Place the plate on a shaker for 15 minutes at room temperature, additional up and down pipetting steps will increase the cell lysis.Freeze cells at -20 or -80°C and thaw them afterwards
- The cell lysate can be placed in storage tubes or measured in the plate by adding reconstructed Renilla GLOW-Juice.

Standard Protocol

Program luminometer:
For the measurement we suggest a delay of 2 sec. after adding the reagent to the lysate and a measuring time of 5 sec..
1.) Transfer 20µl cell lysate into your luminometer tube.
2.) Add 100 µl **Renilla GLOW-Juice**
3.) Start measurement

Standard procedure:



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