

Renilla-Juice SMALL KIT (1 02 530)

Components include:

Renilla-Juice	5 x 10 ml Buffer for measuring Renilla-Luciferase – without substrate. Store at +4°C.
Coelenterazine(CTZ)	5 x 1 vial lyophilised substrate for 10 ml Renilla-Juice (synth.) Coelenterazine for Renilla- and Gaussia- Luciferase Store at -80°C in the dark.
Reconstruction buffer	200 µl for dissolving the lyophilised Coelenterazine Store at -80°C in the dark.
2x Lysis-Juice 2	1 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-Juice SMALL KIT includes 5x 10 ml Test-Systems for Renilla-Luciferase. Note: If the lyophilised Coelenterazine is dissolved in the Reconstruction buffer the stability will decrease after 30 Days. Please pipette the 200 µl Reconstruction buffer into the brown glass tube of lyophilised CTZ. It results 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-Juice **shortly before use** (2µl CTZ into 100 µl Renilla-Juice). The reagents should reach at least room temperature (20-25°C) before starting measuring the luciferases! Reminders of the mixed Renilla-Juice should not be frozen again because it will lose noticeable activity.

Preparation of Cell Lysates:

Renilla-Juice SMALL KIT includes Lysis-Juice 2. This buffer doesn't contain detergent, 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-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-Juice**
- 3.) Start measurement

Standard procedure:

