

## Renilla-Juice BIG KIT (1 02 531)

### Components include:

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

### Reconstruction:

This Renilla-Juice BIG KIT includes 100 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 2 ml Reconstruction buffer into the brown glass tube of lyophilised 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-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 loose noticeable its activity.

### Preparation of Cell Lysates:

Renilla-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:

<b>Culture Plate</b>	<b>Vol. Lysis-Juice</b>
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:

