Genofax B 500

Reporter genes detection - Firefly luciferase & Renilla luciferase

Product Data Sheet

YELEN ANALYTICS

Product Information

Reporter genes detection - Firefly luciferase & Renilla luciferase

Reference: Genofax B 500

Storage Temperature: 2 years at 2-4°C for Firefly Buffer
1 year at -18°C for Luminescent Firefly (reconstituted or not)
1 years at 2-4°C for Renilla Buffer and Renilla Reconstitution
1 year at -18°C for Luminescent Renilla not reconstituted (2 months if reconstituted)

Technical Bulletin & Protocol

Product Description

The Genofax B kit is used to reveal the reporter gene of Firefly luciferase and of the Renilla luciferase in **the same well (or tube)**. The revelation is made according to the principle of bioluminescence described below.

The first reagent allows the detection of the Firefly Luciferase:

$$\text{Luciferin} + \text{ATP} + \text{CoA} + O_2 \xrightarrow[Mg^{2+}]{\textbf{Firefly Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{PPi} + CO_2 + \text{Light}$$

The presence of coenzyme A (CoA) optimizes the stability and sensitivity of the reaction. The quantification of the light emitted is performed in a plate or tube luminometer during a period of 5 to 10 seconds.

The second reagent, in addition to "quenching" the first luminescence reaction, allows the detection of Renilla Luciferase:

$$\text{Cœlenterazine} + O_2 \xrightarrow{\textbf{Renilla Luciferase}} \text{Cœlenteramide} + CO_2 + \text{Light}$$

Under the conditions of use described below and with a suitable luminometer, the response of the Firefly reagent is linear between 10^{-19} and 10^{-14} moles of Firefly Luciferase. The Renilla reagent is linear between 10^{-18} and 10^{-14} moles of Renilla Luciferase.

Components

The kit is sufficient for 500 assays.

1 Firefly luciferase bioluminescent reagent (**Luminescent Firefly**) (1,5 ml vial): ..... storage at -18°C.

1 **Firefly Buffer** solution (50 ml bottle): ..... storage at 2 to 4°C.

1 Renilla luciferase bioluminescent reagent (**Luminescent Renilla**) (1,5 ml vial): ..... storage at -18°C.

1 **Renilla Buffer** solution (50 ml bottle): ..... storage at 2 to 4°C.

1 Reconstitution solution (**Renilla Reconstitution**) (1,5 ml vial): ..... storage at 2 to 4°C.

Reagents and Equipment Required but Not Provided

Luminometer multiwell plate reader, vortex.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.

Preparation Instructions

1. Reconstitute the vial of **Luminescent Firefly** reagent a few minutes in advance with 1 ml of **Firefly Buffer**. Homogenize with vortex vigorously (30 sec) (after use, this solution can be stored at -18°C).
2. Prepare a stock of **Firefly Buffer** solution at the rate of 100 µl of buffer solution per test. Add inside this bottle the **Luminescent Firefly** reagent at a rate of 20 µl per ml of Firefly Buffer solution, in order to form **ready to use Firefly reagent**.
3. At the first use, reconstitute the vial of **Luminescent Renilla** with 600 µl of **Renilla Reconstitution**. Close the vial cap and shake gently for 10 seconds.
4. Make an amber or opaque bottle of **Renilla Buffer** solution at the rate of 100 µl of buffer solution per test. Add inside this stock the **Luminescent Renilla** reagent at a rate of 10 µl per ml of Renilla Buffer solution, in order to form the **ready to use Renilla reagent**.

Caution: Close the Renilla Luminescent vial and quickly replace it under storage conditions.

Procedures

All samples and standards should be run in duplicate or triplicate.

Sample Preparation

Before the luminescent assay reaction, samples have to be lysed with adapted lysis solution.

Luminescent Assay Reaction

1. Add 20 µl of sample to well or tube.

2. Add 100 µl of **ready to use Firefly reagent** to sample well or tube.

3. Immediately measure the luminescence of sample well or tube (recommended measuring time: 1 to 5 seconds).

4. Add 100 µl of **ready to use Renilla reagent** to sample well or tube.

5. Wait 5 to 10 seconds

6. Immediately measure the luminescence of sample well or tube (recommended measuring time: 1 to 5 seconds).

Note: - Reagent background control is obtained by use of 20 µl of non-transfected cell lysate or 20 µl of the lysis solution used instead of the sample.

- The quantification of emitted light is done in a plate or tube luminometer for a duration of 1 to 5 seconds for each luciferase with 5 seconds of "rest" between each quantification in order to optimize the quenching of the Firefly Luciferase.

Contact information

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