Promega

Product Application

P450-Glo™ CYP3A4 Assay on 3D microtissues

Measure cytochrome P450 (CYP) activity in 3D microtissues using the P450-Glo™ CYP3A4 Assay with Luciferin-IPA.

Assay: P450-Glo™ CYP3A4 Assay with Luciferin-IPA (Cat. #V9001)

Analyses: Viability-CellTiter-Glo® 3D Cell Viability Assay (Cat. #G9681)

Sample Type(s): *human liver microtissues*

Input: Single microtissue per assay

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manual #TB325, available at: **www.promega.com/protocols**

Materials Required:

- P450-Glo™ CYP3A4 Assay with Luciferin-IPA (cat. #V9001)
- CellTiter-Glo® 3D Cell Viability Assay (cat. #G9681)
- 1X PBS
- Luminometer, e.g. GloMax® Discover System (Promega cat. #GM3000)
- Plate shaker

Protocol:

Preparation/treatment of microtissues

1. Remove medium from microtissues.
2. Add 50µl of medium with test compound or vehicle control.
3. Incubate for desired time at 37°C, 5% CO_2.

Measurement of cytochrome P450 (CYP) activity (following technical bulletin #TB325)

1. Dilute the P450-Glo™ CYP3A4/Luciferin-IPA substrate to 3µM in LiMM.
2. Remove medium from the microtissues.
3. Wash the microtissues by gently adding 100µl 1X PBS.
4. Remove the PBS and add 50µl of diluted P450-Glo™ substrate.
5. Incubate at 37°C, 5%CO_2 for 60 minutes.
6. During incubation, prepare the Luciferase Detection Reagent (LDR).
7. Non-lytic method
    a. Transfer 25µl of medium to a 96-well white plate.
    b. Add 25µl of LDR to the plate and mix for 1 minute at 600 rpm on a plate shaker.
    c. Incubate the plate at room temperature for 20 minutes, then read luminescence using a luminometer such as the GloMax® Discover System.

    Lytic method (alternative)
    a. Add an equal volume of LDR to each well, and mix briefly on a plate shaker.
    b. Incubate the plate at room temperature for 20 minutes, then read luminescence using a luminometer such as the GloMax® Discover System.

Multiplex CellTiter-Glo® 3D Cell Viability Assay with P450-Glo™ Assay (use with Non-lytic method only)

1. To the remaining 25µl of media/microtissue, add an equal volume (25µl) of room temperature CellTiter-Glo® 3D Reagent (see technical manual #TM412 for reagent preparation).
2. Shake plate for 5 minutes at 600rpm using a plate shaker.
3. Incubate plate for another 25 minutes at room temperature.
4. Remove an aliquot (such as 40µl) to an opaque white luminometer plate to measure luminescence.

Results:

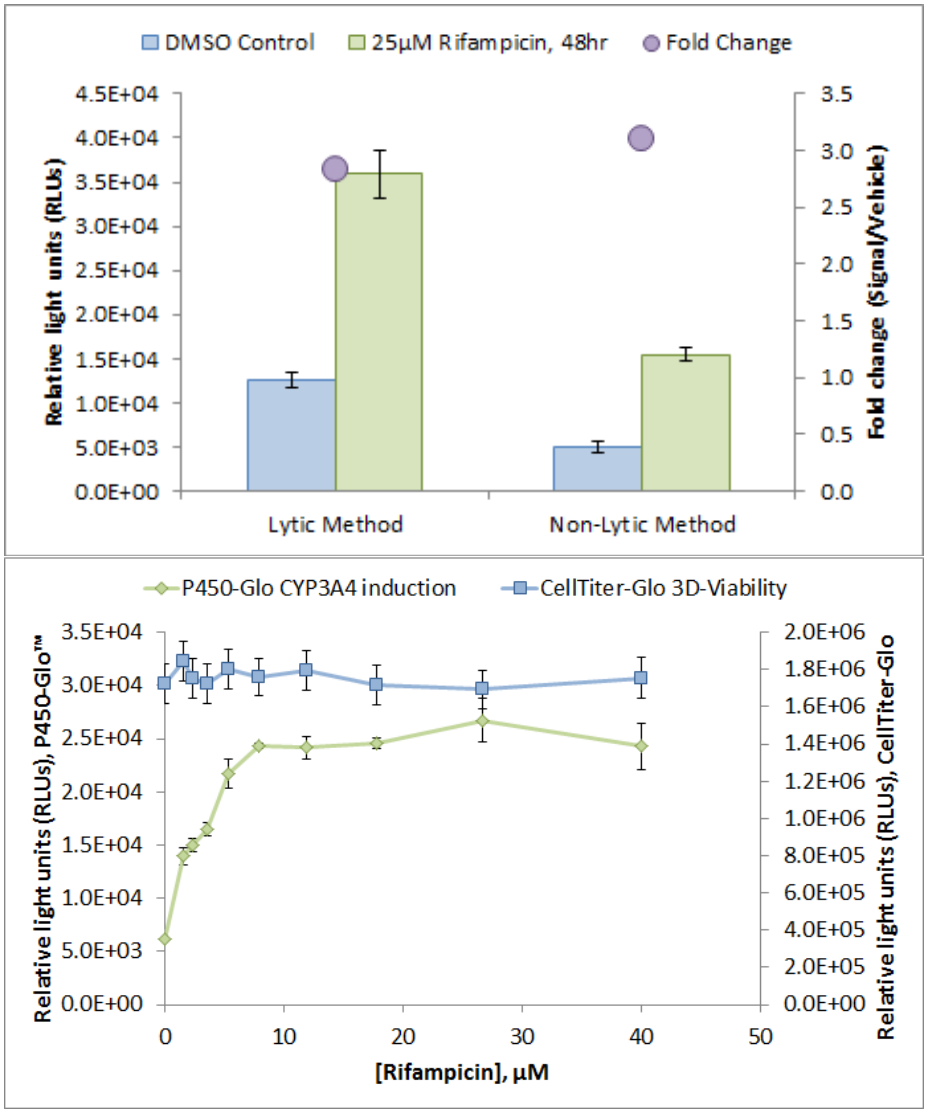

Figure: Top panel – Human liver microtissues were dosed with 25µM Rifampicin (Sigma, cat. #R3501) or vehicle control (0.1% DMSO) for 48 hours followed by detection of CYP3A4 using the P450-Glo™ Assay

(n=3). Both the lytic and non-lytic methods were used to measure induced cytochrome P450 activity. The fold increase in activity over the DMSO control is indicated on the graph. NOTE: The non-lytic method only measures half of the total available signal. **Bottom panel -** Human liver microtissues were dosed with serial dilution of Rifampicin for 48 hours followed by detection of CYP3A4 using the P450-Glo™ Assay (non-lytic method, n=3). Viability was measured on the same samples using the CellTiter-Glo® 3D Cell Viability Assay.
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