P450-Glo™ CYP3A4 Assay with Luciferin-IPA for Cell-Based Samples

Instructions for Use of Products V9001and V9002.

Quick Protocol

The P450-Glo™ CYP3A4 Assay with Luciferin-IPA substrate can be used to selectively measure CYP3A4 activity in cells. Significant activity of other human CYPs is not detected with Luciferin-IPA substrate.

These instructions are for cell-based assays performed in a 96-well plate formats. For formats other than a 96-well plate, reagent volumes should be scaled proportionately. For detailed instructions, including plate setup, see the *P450-Glo™ Assays Technical Bulletin* #TB325, available at: **www.promega.com/protocols/**

After cell culture and compound treatment steps are completed (see section below), there are two protocol options for measuring CYP3A4 activity with this assay. Select the protocol that meets your experimental needs.

a. **Nonlytic Protocol:** Medium is removed from the cell culture plate and transferred to another plate, where the assay is performed. This protocol preserves cells for downstream analysis.
b. **Lytic Protocol:** Assay is performed in-well with cells. This protocol streamlines the workflow.

Table 1. Volumes Required for P450-Glo™ CYP3A4 Cell-Based Assay.

	Nonlytic Protocol	Lytic Protocol
Sample (96-well format)	25µl transferred medium	50µl in well with cells
Luciferin Detection Reagent	25µl	50µl

Notes:

a. Reserve some empty wells (no cells) for background measurements.
b. See the *P450-Glo™ Assays Technical Bulletin* #TB325, for cell culture recommendations.
c. If using the Lytic protocol, cells should be cultured in white-walled, collagen-coated culture plates with clear bottoms (e.g., BioCoat® 96-well plates, Corning Cat.# 354650/356650/356701) to avoid crosstalk between wells.

Cell, Treatment and Reagent Preparation for the P450-Glo™ CYP3A4 Assay

1. Treat cells with test compounds. For CYP gene induction studies, cells are typically treated with inducers for 24–72 hours. Optimal treatment time should be determined empirically; however, 48 hours is a common starting point. See Table 2. for examples of CYP gene inducers that can be used as positive controls. Change medium with test compounds, etc., once daily for the duration of the treatment time.

Table 2. Common CYP3A4 Gene Inducers.

Inducers	Pathway
25µM rifampicin	pregame X receptor (PXR)
500µM phenobarbital	constitutive androstane receptor (CAR)
50µM dexamethasone	glucocorticoid receptor (GR)

2. After treatment is completed, thaw the luminogenic substrate, Luciferin-IPA. Keep at room temperature until use
   Note: Store excess substrate at –30°C to –10°C and protect from light.
3. Prepare 50µl fresh culture medium containing 3µM Luciferin IPA (1:1,000 dilution) for each well of a 96-well plate.

Cell, Treatment and Reagent Preparation for the P450-Glo™ CYP3A4 Assay (continued)

4. Replace culture medium with 50µl of prepared medium containing Luciferin-IPA.

   Optional: Wash cells with medium or phosphate-buffered saline before adding medium with substrate. Some compounds that induce CYP gene expression also inhibit the CYP enzyme activity. To observe induction, you may need to remove the inducer by including a wash step prior to adding a luminogenic substrate.

   Notes:

   a. If inhibitors of basal or induced CYP enzyme activity are being tested, add them with the luminogenic substrate.

   b. Consider including an inhibition control for induced P450 activity with a selective P450 inhibitor, such as 1µM ketoconazole, to confirm that the induced activity is from CYP3A4.

5. To determine background luminescence, add medium with 3µM Luciferin-IPA to a set of empty wells (no cells).

6. Incubate plates at 37°C for 30–60 minutes.

7. During incubation, prepare the Luciferin Detection Reagent.

   a. Equilibrate the lyophilized Luciferin Detection Reagent and the Reconstitution Buffer with Esterase to room temperature.

   b. Transfer the contents of one bottle of reconstitution buffer to one amber bottle containing the lyophilized Luciferin Detection Reagent. Mix by swirling or inverting several times to obtain a homogeneous solution. Store at room temperature until ready to use.

   Note: The reconstituted Luciferin Detection Reagent can be stored at room temperature for 24 hours or at +2°C to +10°C for 1 week without loss of activity. For long-term storage, store at –30°C to –10°C for up to 3 months. Be sure to mix the thawed Luciferin Detection Reagent well before use.

P450-Glo™ CYP3A4 Assay with Luciferin-IPA for Cell-Based Samples

Quick Protocol

Instructions for Use of Products V9001and V9002.

Nonlytic P450-Glo™ CYP3A4 Assay using Cultured Cells in Monolayer

1. Transfer 25µl of culture medium from each well to a 96-well opaque white luminometer-compatible plate at room temperature, and add 25µl of Luciferin Detection Reagent to initiate a luminescent reaction.
2. Incubate the plate at room temperature for 20 minutes, then read luminescence.
   Note: Luminometer settings will depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.
3. Calculate net signals by subtracting background luminescence values (no-cell control) from test compound-treated and untreated (vehicle control) values.
4. Calculate percent change by dividing net treated values by net untreated values and multiplying by 100.
   Optional: Perform the CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7570, G7571, G7572 and G7573) to normalize P450-Glo™ Assay values to cell number.

Lytic P450-Glo™ CYP3A4 Assay using Cultured Cells in Monolayer

1. Add 50µl of Luciferin Detection Reagent to each well, and mix briefly on a multiwell plate shaker or by gently tapping or swirling the plate to form a lysate.
2. Equilibrate the plate at room temperature for 20 minutes.
   Note: Luminescence from lysates of some, but not all, cell types may decay rapidly, so it might be necessary to record luminescence within 20 minutes of adding Luciferin Detection Reagent.
3. Read luminescence.
   Note: Luminometer settings will depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.
4. Calculate net signals by subtracting background luminescence values (no-cell control) from test compound-treated and nontreated (vehicle control) values.
5. Calculate percent change by dividing net treated values by net nontreated values and multiplying by 100.

PROMEGA CORPORATION · 2800 WOODS HOLLOW ROAD · MADISON, WI 53711-5399 USA · TELEPHONE 608-274-4330
WWW.PROMEGA.COM · ©2024 PROMEGA CORPORATION · ALL RIGHTS RESERVED · PRICES AND SPECIFICATIONS SUBJECT TO CHANGE WITHOUT PRIOR NOTICE · PRINTED IN USA 5/24 · PART #FB249