

# Protocol of P450-Glo™ CYP3A4 Assay for High-throughput Screening

**DOCUMENT:** P450-Glo™ CYP3A4\_TOX21\_SLP\_Version1.0

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## ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
CYP3A4	N/A	Human	Enzyme	Luminescence	Promega Corporation	Cell-free cytochrome P450

## QUALITY CONTROL PRECAUTIONS:

1. Avoid repeated freeze-thaw cycles of substrate and enzyme.
2. Dispense the unused Luciferin-PPXE and CYP3A4 membranes into single-use aliquots and store at -70°C.
3. Keep the mixtures on ice during assay run.

## MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-P450-Glo™ CYP3A4 Screening System (Luciferin-PPXE) DMSO Tolerant Assay	-Promega	-Promega/V9910
-Ketoconazole (Positive control compound)	-Sigma Aldrich	-Sigma Aldrich/K1003
-1536-well medium binding white solid plates	-Greiner Bio-one	-Greiner Bio-one/789175-F
-BioRAPTR FRD	-Beckman Coulter	-Beckman Coulter
-Pintool station	-Wako	-Wako
-ViewLux plate reader	-Perkin Elmer	-Perkin Elmer

## PROCEDURE:

### 1. Enzyme-substrate mixture:

- i. 100mM Tris-HCl = 11.975uL
- ii. 50mM Luciferin-PPXE = 0.025uL
- iii. CYP3A4 membranes = 0.5uL
- iv. 0.2% BSA (7.5% stock) = 0.67uL
- v. Luciferin-Free water = 11.83uL
- vi. Final volume = 25.0uL

### 2. NADPH regeneration solution:

- i. Luciferin-Free water = 12.0uL
- ii. Potassium Phosphate Buffer, 1M = 10.0uL
- iii. Solution A = 2.5uL
- iv. Solution B = 0.5uL
- v. Final Volume = 25.0uL

### 3. Assay Protocol

- i. Two uL of enzyme-substrate mixture was dispensed dispenser in 1536-well medium-binding white solid plates using BioRAPTR dispenser.
- ii. The positive control and test compounds were transferred at 23nL to 1-4 and 5-48 columns of the assay plates respectively using Pintool station.

Positive control plate format: Column 1: two-fold sixteen-point titration starting at 10mM (final = 57.5uM) duplicates; Columns 2 & 3 (top halves): 1.25mM (final = 7.2uM) and 625uM (3.6uM) Ketoconazole respectively; Columns 2 & 3 (bottom halves) and 4: DMSO.

Control used is Ketoconazole [10mM made in DMSO (final = 57.5uM)]

- iii. The assay plates were incubated for 10min at room temperature.
- iv. The reaction was initiated through the addition of 2uL NADPH regeneration solution using BioRAPTR dispenser.
- v. The assay plates were incubated for 60min at room temperature to allow the reaction to continue before it was stopped with a detection reagent.
- vi. Four uL of detection reagent was added using BioRAPTR dispenser.
- vii. The assay plates were incubated for 20min at room temperature.
- viii. The luminescence intensity was quantified using ViewLux plate reader (Exposure time: 60sec).

## ASSAY PERFORMANCE:

Online Validation	P450-Glo™ CYP3A4 assay
AC <sub>50</sub> (uM)	0.02 ± 0.001 (n = 26)
CV*	2.13 ± 0.36 (n = 27)
S/B	4.36 ± 0.05
Z	0.88 ± 0.05

\*CV values shown represent average of DMSO column from each plate.