CYP2C9 Induction Reporter Assay Kit

Item No. 601120

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
3 Safety Data
4 Precautions
4 If You Have Problems
4 Storage and Stability
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
6 About This Assay

ASSAY PROTOCOL
7 Plate Set Up
9 Performing the Assay

ANALYSIS
10 Calculations
11 Performance Characteristics

RESOURCES
12 Troubleshooting
13 References
14 Plate Template
15 Notes
16 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	100 Tests Quantity/Size	Storage
601121	CYP2C9 Reporter Reverse Transfection Strip Plate	1 plate	-20°C
601122	Dexamethasone Positive Control (10 mM)	1 vial/10 µl	-20°C
600183	SEAP Substrate (Luminescence)	1 vial/15 ml	4°C
700029	96-Well Solid Plate (white)	3 plates	RT
400012	96-Well Cover Sheet	3 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

Remove the Dexamethasone Positive Control from the kit and store at -20°C. Store the CYP2C9 Reporter Reverse Transfection Strip Plate at -20°C. The SEAP Substrate should be stored at 4°C. The kit will perform as specified if used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. C3A cells or HepG2 cells; both cell lines can be obtained from ATCC
2. Culture medium used for the cells (MEM containing 10% Fetal Bovine Serum (FBS))
3. A plate reader capable of measuring luminescence
4. Adjustable pipettes and a repeating pipettor
5. An incubator set at 65°C
6. Penicillin-Streptomycin (100X) (Invitrogen 15140-122)

INTRODUCTION

Background

The cytochrome P450s (CYPs) are heme-thiolate monooxygenases that metabolize a wide range of endogenous compounds and xenobiotics, such as pollutants, environmental compounds, and drugs.[1] These enzymes are essential for the metabolism of many medications and their induction is one of the factors that can affect the pharmacokinetics of a drug upon multiple dosing. In particular, CYP2C9 is the principal member of the CYP2C subfamily of P450 enzymes in the human liver. It is responsible for metabolizing approximately 20% of all prescribed drugs, including the diabetic agent tolbutamide, the anticonvulsant phenytoin, the anticoagulant warfarin, numerous anti-inflammatory drugs such as ibuprofen and diclofenac, the antihypertensive losartan, and many others.[2] Xenobiotic induction of the human CYP2C9 gene is regulated at the transcriptional level through interaction with nuclear receptors such as PXR, CAR, HNF4α, and GRα. Strong induction of CYP2C9 is mediated by the human Glucocorticoid Receptor (GRα) directly binding to the glucocorticoid response element (GRE) in the promoter region of the gene.[3,4] Early screening of drug candidates for induction or inhibition of CYP2C9 is a critical step in preventing potential drug failure due to toxicity or low efficacy at the later stages of drug development. Cell-based reporter assays that typically involve cultured cell lines transfected with a reporter gene construct containing a promoter/enhancer sequence of the enzymes have provided a means of high-throughput enzyme induction assessment. Therefore, a cell-based reporter assay to evaluate hGR-mediated CYP2C9 induction represents a novel assay for the discovery of hepatic CYP inducers.

About This Assay

Cayman's Reverse Transfection Reporter Assays have overcome many of the disadvantages of other transfection approaches. In this method, a proprietary transfection complex containing DNA and an optimized mixture of lipids and proteins is evenly applied and processed on the culture surface of multi-well plates. Adherent cells, supplied by the user, are applied directly to the plate and allowed to grow in the coated wells. Using this method, the uptake of the DNA complex by the cell increases dramatically compared to solution-phase transfection, enhancing both the transfection efficiency and the co-transfection efficiency for multiple plasmids.

Cayman's CYP2C9 Induction Reporter Assay Kit consists of a 96-well plate coated with a transfection complex containing a Secreted Alkaline Phosphatase (SEAP) reporter regulated by the human CYP2C9 gene promoter. The complex also contains two additional expression constructs, the transcriptionally active isoform of the human Glucocorticoid Receptor (GRα) and human Hepatic Nuclear Factor 4α (HNF4α). Cells grown on the CYP2C9 Reporter Reverse Transfection Strip Plate will introduce the reporter gene and express GRα as well as HNF4α. Binding of inducer-activated transcription factors to the CYP2C9 promoter initiates a signal resulting in expression of SEAP, which is secreted into the cell culture medium. Aliquots of medium are collected at time intervals beginning at about 24 hours and SEAP activity is measured simply by adding a luminescence-based alkaline phosphatase substrate provided in the kit. The kit is simple to use and can be easily adapted to high-throughput screening for potential GR-mediated CYP2C9 inducers. A known CYP2C9 inducer, dexamethasone, is included in the kit for use as a positive control. The kit provides sufficient reagent to measure SEAP activity at three time points using the white plates provided.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical experimental plate will include wells with cells treated with dexamethasone provided in the kit (positive control), wells with cells treated with experimental compounds, and wells of untreated cells. We recommend that each treatment be performed at least in triplicate. Record the contents of each well on the template sheet provided on 14.

Addition of Cells to the Reverse Transfection Plate

IMPORTANT

Before starting the experiment, dilute Penicillin-Streptomycin (100X, Invitrogen 15140-122) 1:100 in culture medium used for your cells. This will be the culture medium for your experiment.

1. Remove the unopened CYP2C9 Reporter Reverse Transfection Strip Plate (Item No. 601121) from the freezer and allow to equilibrate to room temperature. Clean the bag with 70% alcohol before opening the bag. Place the plate in the hood and remove from the bag.

   *NOTE: If you are not using the whole plate at one time for your experiment, remove the number of strips needed, put the remaining strips back in the bag, and store in a desiccator, **protected from UV light**, at room temperature for up to a week. Alternatively, you can vacuum seal the bag and store the remaining strips at -20°C for up to two months.*

2. Seed each well of the plate at a density of 40,000-60,000 cells/well in 150 µl of culture medium. Place the plate in a 37°C cell incubator and incubate for 24 hours.

Cell Stimulation

1. After 24 hours of incubation, check the cells for confluency.
2. Prepare test compounds at 4X the desired final concentration in culture medium, as prepared above, and pipette 50 µl to the assigned wells. Wells containing untreated cells receive 50 µl of culture medium only. For positive controls using the provided dexamethasone, dilute the 10 mM Dexamethasone Positive Control (Item No. 6001122) 1:10,000 in the culture medium and add 50 µl to corresponding wells. At this concentration, dexamethasone induces a 20- to 100-fold increase in SEAP activity, depending on the cell type and stimulation.
3. Return the plate to 37°C incubator.

NOTE: It is recommended the assay be performed when the cells are near confluency. For prolonged incubations, test compounds may need to be replenished daily with media change.

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Before performing the assay, remove the SEAP Substrate (Luminescence) (Item No. 600183) from the refrigerator and allow to equilibrate to room temperature.

1. After 24-72 hours of stimulation with test compounds and dexamethasone, collect 10 µl of medium from each well and transfer into a 96-Well Solid Plate (white) (Item No. 700029) inside a culture hood. The Reverse Transfection Strip Plate must be returned into the incubator if further sample collection will be done. *NOTE: It is recommended to collect samples for assay after 24, 48, and 72 hours of stimulation.*
2. Cover the white plate with the lid and inactivate endogenous alkaline phosphatase by heating the samples at 65°C for 30 minutes. The SEAP expressed in this assay is stable under these conditions.
3. Remove the plate from the 65°C incubator and allow to equilibrate to room temperature.
4. Add 50 µl of substrate to each well, shake briefly, and incubate the plate at room temperature for 30 minutes.
5. Read the plate with a plate reader capable of detecting a luminescent signal.

NOTE: The plate should be read immediately after incubation. When multiple plates are processed at the same time, the addition of substrate and reading of the plate should be done plate by plate in order to allow equal time from addition of substrate to the time the plate is read.

ANALYSIS

Calculations

According to the industry guidelines, a compound that produces a change that is equal to or greater than 40% of the positive control can be considered as an enzyme inducer *in vitro* and therefore evaluation *in vivo* is warranted.

For each compound, calculate the % Response as follows:

$$\% \text{ Response at X Concentration} = \left[\frac{\text{(SEAP activity (RLU) of test drug treated cells) - (SEAP activity (RLU) untreated cells)}}{\text{(SEAP activity (RLU) of dexamethasone treated cells) - (SEAP activity (RLU) untreated cells)}} \times 100 \right]$$

Performance Characteristics

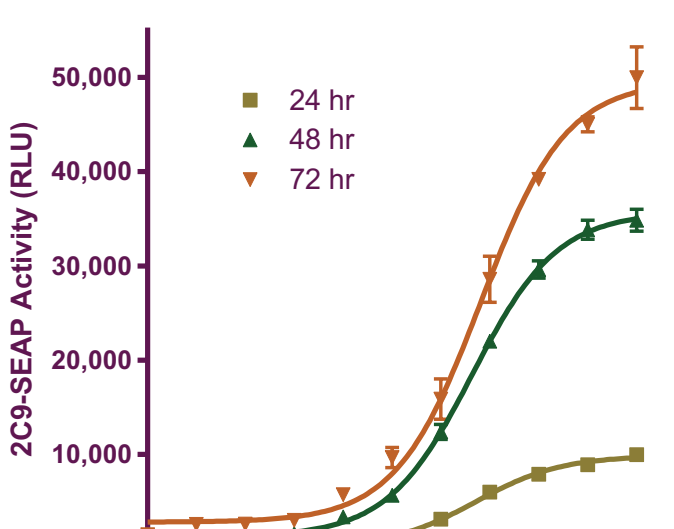


Figure 1. GRα-mediated CYP2C9 SEAP reporter activity in HepG2 cells in response to dexamethasone stimulation. HepG2 cells were plated on a CYP2C9 Reporter Reverse Transfection Strip Plate at a density of 50,000 cells/well. At 24 hours after plating, cells were treated with different doses of dexamethasone as indicated above on the x-axis. After 24, 48, and 72 hours of stimulation, 10 µl of culture media was removed from each well and assayed for SEAP activity according to the protocol described on 9. Z'=0.78 at 48 hours

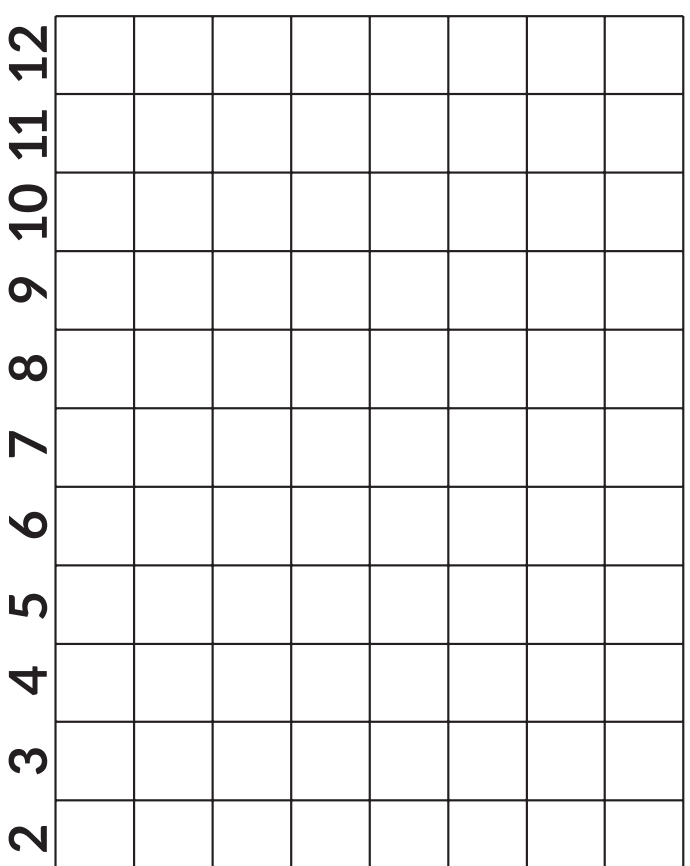
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Erratic response curve of test compounds	Unequal number of cells in each well	Make sure each well contains the same number of cells
High reading in all wells	Cell density is too high or treatment was too long	Plate cells more sparsely (decrease treatment time)
Decrease in SEAP activity at high doses of compound	Cytotoxicity at high doses of compound	Use compound at low doses

References

1. Tanaka, E. Clinically important pharmacokinetic drug-drug interactions: Role of cytochrome P450 enzymes. *J. Clin. Pharm. Ther*. **23(6)**, 403-416 (1998).
2. Goldstein, J.A. and de Morais, S.M. Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* **4(6)**, 285-299 (1994).
3. Gerbal-Chaloin, S., Daujat, M., Pascussi, J.M., *et al*. Transcriptional regulation of CYP2C9 gene. Role of glucocorticoid receptor and constitutive androstane receptor. *J. Biol. Chem*. **277(1)**, 209-217 (2002).
4. Dvorak, Z. and Pavek, P. Regulation of drug-metabolizing cytochrome P450 enzymes by glucocorticoids. *Drug Metab. Rev*. **42(4)**, 621-635 (2010).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©05/08/2026, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.