CYP1A1/2 Induction Reporter Assay Kit

Item No. 600670

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

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
600672	CYP1A1/2 Reporter Reverse Transfection Strip Plate	1 plate	-20°C
600671	3-Methylcholanthrene Positive Control	1 vial/40 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: 734-971-3640

Email: techserv@caymanchem.com

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 3-Methylcholanthrene Positive Control from the kit and store at -20°C (be careful to avoid repeated freeze/thaw cycles). Store the CYP1A1/2 Reporter Reverse Transfection Strip Plate at -20°C. The SEAP Substrate should be stored at 4°C and will be stable for at least one year. 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. This can result in pharmacokinetic drug-drug interactions with co-administrated drugs, causing potential therapeutic failures. Of more than 50 known CYP enzymes, five of them, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 metabolize more than 90% of drugs. CYP1A1 and CYP1A2 are highly inducible human CYPs which are involved in the detoxification of xenobiotics such as pharmaceutical drugs and environmental pollutants as well as metabolic activation of these compounds.[2-3] Thus, polycyclic aromatic hydrocarbons found in cigarette smoke and other products of combustion, as well as arylamines, such as heterocyclic amines found in char-broiled meat, undergo metabolic activation by the CYP1A enzymes to produce highly reactive intermediates that possess mutagenic and carcinogenic activities. Early screening of a drug candidate's potency to induce or inhibit CYP1A1/2 is a critical step in preventing potential drug failure due to toxicity or low efficacy at the later stages of drug development.

Primary human hepatocytes are commonly used for assessment of CYP1A2 induction. However, their limited supply and significant donor-to-donor variation complicate their application in early drug discovery. Consequently, there is a need to generate human hepatocyte-like cells which are unlimited in supply and provide the regulatory pathways involved in drug metabolism. 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.

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 CYP1A1/2 Induction Reporter Assay Kit consists of a 96-well plate coated with a transfection complex containing a Secreted Alkaline Phosphatase (SEAP) reporter construct regulated by the human CYP1A1/2 gene promoter.[4] Cells grown on the CYP1A1/2 Reverse Transfection Strip Plate will introduce the reporter gene into the nucleus. Binding of inducer-activated endogenous transcription factors to the CYP1A1/2 promoter initiates a signal resulting in expression of SEAP which is secreted into the cell culture medium. Aliquots of medium are removed 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 CYP1A1 and CYP1A2 inducers. A known CYP1A1/2 inducer, 3-methylcholanthrene or 3-MC, 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 3-MC 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 page 18.

Addition of Cells to the Reverse Transfection Plate

IMPORTANT

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

1. Remove the CYP1A1/2 Reporter Reverse Transfection Strip Plate (Item No. 600672) 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 with C3A or HepG2 cells at a density of 30,000-50,000 cells/well in 200 µl of culture medium. Place the plate in a 37°C incubator and incubate overnight or up to 48 hours.

Cell Stimulation

1. After 24 hours of incubation, aspirate the culture media from each well.
2. Add 100 µl of culture media to each well.
3. Prepare test compounds at 2X the desired final concentration in the above culture medium and pipette 100 µl to the assigned wells. Wells containing untreated cells receive 100 µl of culture medium only. For positive controls using the provided 3-MC, dilute the 3-Methylcholanthrene Positive Control (Item No. 600671) 1:500 in the culture medium and add 100 µl to corresponding wells. At this concentration, 3-MC induces a 10-15 fold increase in SEAP activity, depending on the cell type and stimulation time used.

NOTE: It is recommended the assay be performed when the cells are near confluency.

Performing the SEAP 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 refrigerator and allow to equilibrate to room temperature.

1. After 24-72 hours of stimulation with test compounds and 3-MC, 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 *in vivo* evaluation is warranted.

For each compound, calculate the % Response as follows:

$$\left[\frac{\text{(SEAP activity (RLU) of test drug treated cells) - (SEAP activity (RLU) of untreated cells)}}{\text{(SEAP activity (RLU) of 3-MC treated cells) - (SEAP activity (RLU) of untreated cells)}}\right] \times 100$$

Performance Characteristics

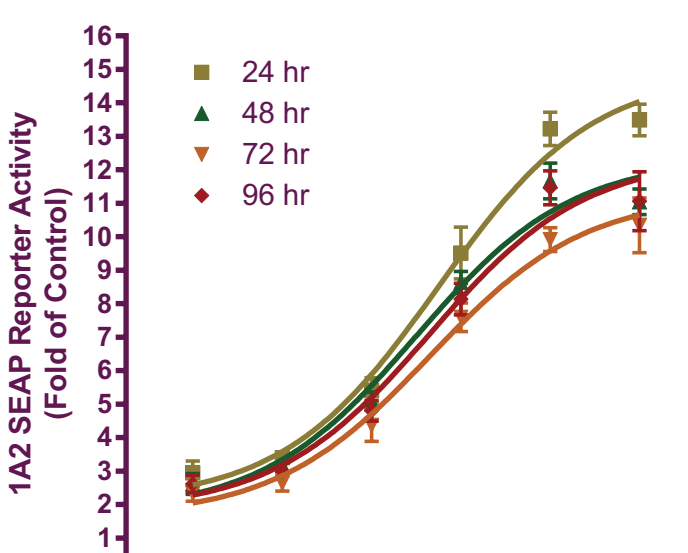


Figure 1. CYP1A1/2 SEAP reporter activity in HepG2 cells in response to 3-MC stimulation. HepG2 cells were plated on a CYP1A1/2 SEAP Reporter Reverse Transfection Strip Plate at a density of 35,000 cells/well. At 24 hours after plating, cells were treated with different doses of 3-MC as indicated above on the x-axis. After 24, 48, 72, and 96 hours of stimulation, 10 µl of culture media was removed from each well and assayed for SEAP activity according to the protocol described on page 9.
NOTE: Z' of the assay at 48 hours is 0.76.

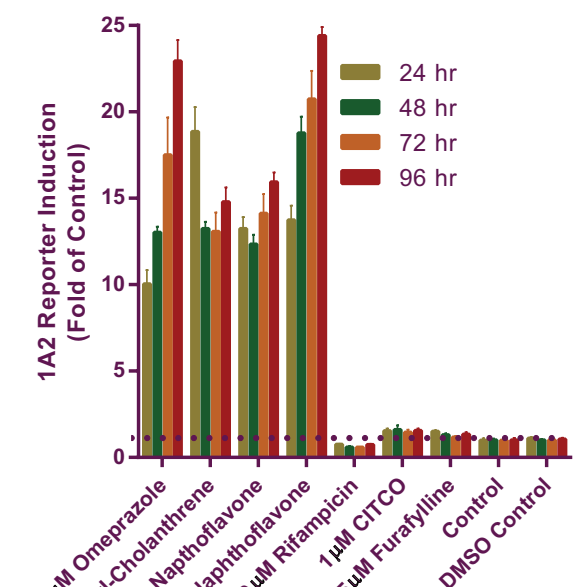


Figure 2. CYP1A1/2 SEAP reporter activity in HepG2 cells in response to a panel of known CYP inducers. Following the protocol indicated in Figure 1, several prototypical CYP inducers were tested at the concentrations indicated in the x-axis. As expected, only the CYP1A1/2 inducers showed a robust response at the different testing times. Data were analyzed by ANOVA followed by Dunnett's multiple comparison versus control. **p <0.01.

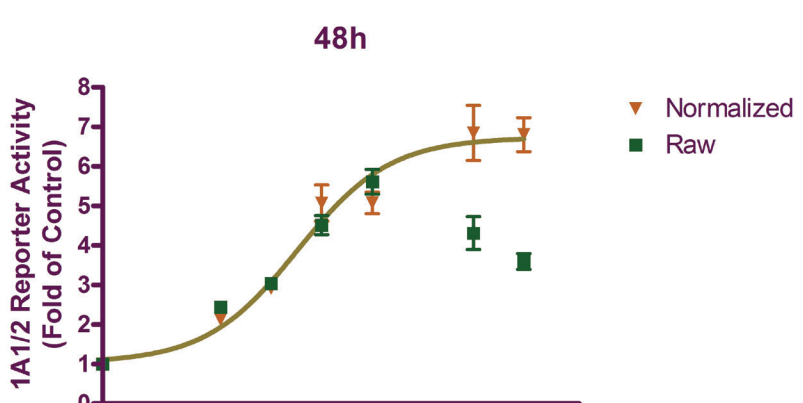


Figure 3. Normalization of cytotoxic responses of 3-MC with WST-1 cell proliferation assay kit. Since some compounds may be toxic to cells at high concentrations, Cayman offers a WST-1 Cell Proliferation Assay Kit (Item No. 10008883) to normalize the induction response at toxic drug concentrations. Shown here is CYP1A1/2-SEAP reporter assay results obtained after 48 hours induction with 3-MC. Raw data (green squares) and normalized data (tan curve) are shown.

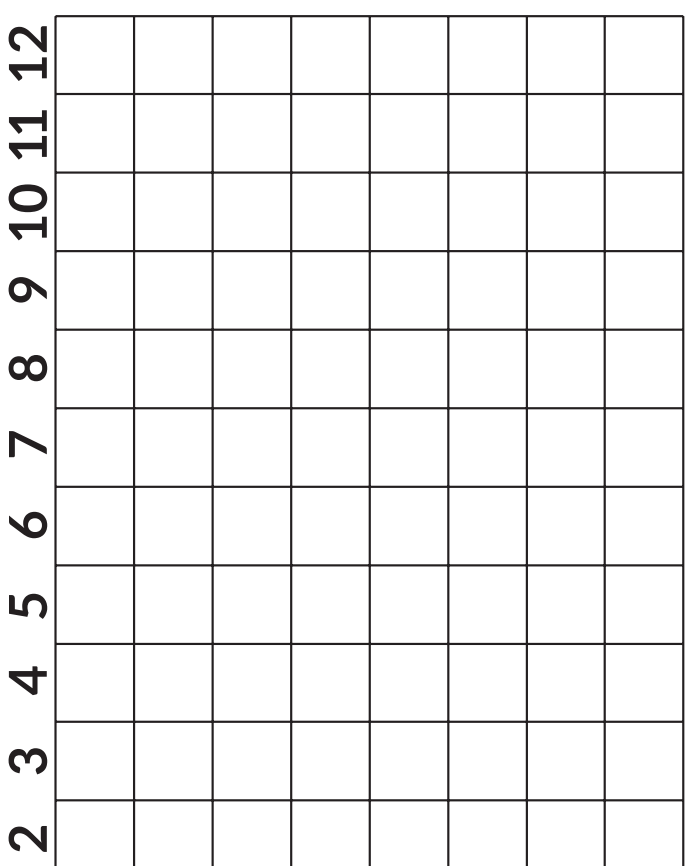
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	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 compound treatments	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	Plate cells more sparsely
Decrease in SEAP activity at high doses of compound	Cytotoxicity at high doses of compound	Use compound at lower doses

References

1. Bibi, Z. Role of cytochrome P450 in drug interactions. *Nutr. Metab.* **5(27)**, (2008).
2. Quattrochi, L.C., Vu, T., and Tukey, R.H. The human CYP1A2 gene and induction by 3-methylcholanthrene. *J. Biol. Chem* **269(9)**, 6949-6954 (1994).
3. Yoshinari, K., Ueda, R., Kusano, K., *et al.* Omeprazole transactivates human CYP1A1 and CYP1A2 expression through the common regulatory region containing multiple xenobiotic-responsive elements. *Biochem. Pharmacol.* **76**, 139-145 (2008).
4. Ueda, R., Iketaki, H., Nagata, K., *et al.* A common regulatory region functions bidirectionally in transcriptional activation of the human CYP1A1 and CYP1A2 genes. *Mol. Pharmacol.* **69(6)**, 1924-1930 (2006).



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.

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