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Induction of Cytochrome P450 subtypes 1A2, 2B6, and 3A4 using Primary Human Interaction Qualified Hepatocytes

Technical information & instructions for enzyme activity assessment

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I. Introduction

This protocol is suitable for the induction of CYP1A2, CYP2B6, and CYP3A4 using Interaction Qualified human hepatocytes. Please read this entire protocol before attempting this procedure. The health of the hepatocytes and induction results are dependent upon following the protocol carefully.

This induction method assesses the potential of test compounds to induce the activity of three specific CYPs: 1A2, 2B6 and 3A4. After 3 days of induction, substrates for each CYP are incubated with the hepatocytes and metabolite formation is measured via LC-MS to determine CYP induction.

For answers to frequently asked questions and citations regarding these products, please visit our knowledge center:

https://knowledge.lonza.com

II. Required reagents and materials

(Components sold separately)

- Cryopreserved Interaction Qualified Hepatocytes (HUCPI, HUCPI-Y)
- Hepatocyte plating medium (MP250-1, MP250-2)
- Hepatocyte Culture Medium Bullet Kit™ (HCM™, CC-3198)
- Omeprazole
- Phenobarbital
- Rifampicin
- Phenacetin
- Testosterone
- Bupropion
- Dimethyl sulfoxide (DMSO)
- RLT Buffer
- Wide bore pipets and pipet tips
- Automated pipettor and serological pipet
- 0.4% solution of Trypan Blue
- Collagen coated cell culture plates (e.g. Corning™ BioCoat™ Collagen I Multiwell Plates; for plated cells only)

III. Preparation of hepatocytes (day 0)

Thaw and plate cryopreserved hepatocytes following Lonza's protocol, which can be downloaded via:

https://bioscience.lonza.com

*Note: Subsequent instructions are for a 24-well plate format. Please adjust volumes according to your specific application or size.

IV. Preparation of induction stock solutions (day 0)

1. Prepare 50 mM Omeprazole (OMZ) stock, 1000 mM Phenobarbital (PHB) stock and 10 mM Rifampicin (RIF) stock by dissolving chemicals in DMSO.
2. After creating stocks, aliquot 50 – 100 µL of each stock into microcentrifuge tubes and store at -20°C for up to 1 year.

V. Preparation of substrate stock solutions (day 0)

1. Prepare 100 mM Phenacetin (PHE) stock, 200 mM Testosterone (TST) stock, and 250 mM Bupropion (BUP) stock by dissolving chemicals in DMSO.
2. After creating stocks, aliquot 50 – 100 µL of each stock into microcentrifuge tubes and store at -20°C for up to 1 year.

VI. Preparation of culture media (day 0)

1. Decontaminate external surfaces of medium bottle with ethanol or isopropanol.
2. To formulate HCM™ Medium, transfer the contents of the HCM™ SingleQuots™ Kit (Catalog No. CC-4182) to HBM™ Basal Medium (Catalog No. CC-3199) with a pipette and rinse each SQ vial with medium. Supplemented medium can be stored at 4°C in the dark for up to 1 month.
3. On each day of treatment (Day 1 - 4), pre-warm supplemented medium to 37°C.

VII. Preparation of induction working solutions (day 1 - 3) and CYP induction

1. Remove stocks of induction compounds from freezer and thaw at room temperature.
2. Label 3 sterile 50 mL conical tubes with each of the 3 inducers (OMZ, PHB, RIF) and a fourth 50 mL conical as DMSO control.
3. Prepare working solutions of each inducer and DMSO control by diluting stock solutions and 100% DMSO 1000-fold in pre-warmed HCM™ Medium.
4. Aspirate HCM™ Medium from all wells of the hepatocyte culture plate and add 500 µL DMSO control and 500 µL working induction solutions to appropriate wells (see example plate layout in figure 1).
5. Return hepatocytes to incubator.

NOTE: Induction dosing should occur within a 24±2 hour time frame. This needs to be taken into consideration on the first day of dosing.

6. Repeat steps 1 - 5 for day 2 and 3 of culture.
7. *Optional mRNA collection*: On day 2 or 3 of treatment, remove media and wash wells with 2X PBS. Add RLT buffer to each well and scrape cells into RLT buffer within the well. Place plate at - 80°C until samples are needed for mRNA analysis.

	1	2	3	4	5	6
A	Control (DMSO only)			OMZ (1A2)	PHB (2B6)	RIF (3A4)
B						
C						
D						

Figure 1. Sample 24-well plate layout with induction compounds.

VIII. Preparation of substrate working solution (day 4)

1. Remove stocks of substrates from freezer and allow to thaw at room temperature.
2. Label 3 sterile 50 mL conical tubes with each of the 3 substrates (PHE, BUP, TST).
3. Prepare working solutions of each substrate by diluting stock solutions 1000-fold in pre-warmed HCM™ Medium.

IX. Enzyme assay (day 4)

1. Aspirate medium from wells and wash cells with warmed HCM™ Medium.

2. Replace medium with 300 µL of appropriate substrate solutions per well, as shown in Figure 2.

NOTE: The substrate must match the CYP inducer according to Figure 2.

3. After adding the substrates, place plate on orbital shaker in 37°C, humidified CO_2 incubator. Shake plate at 95 RPM for incubation times specified in Figure 2.
4. At time points shown in Figure 2, collect 250 µL of well contents and place into appropriate wells of a 96-well block on dry ice to stop the reaction. Alternatively, a stop solution can be used instead of dry ice to stop the reaction
5. After reaction is stopped (via freezing or stop solution in step 4 above), samples are ready for metabolite detection via LC-MS analysis.

Inducer Day1-3		Control (DMSO only)			OMZ	PHB	RIF
		1	2	3	4	5	6
Substrate Day 4	A	PHE	BUP	TST	PHE	BUP	TST
	B						
	C						
	D						
Incubation Time		15min	20min	15min	15min	20 min	15 min

Figure 2. Substrate dosing plate shown in 24-well plate.

X. Product warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of cells only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

When placing an order or for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Primary Cell Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or want to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone or mail. (See Page 1 for details).