General Protocol usingTruVivo™ for Cytochrome P450 Induction Evaluation

TECHNICAL BULLETIN

Purpose

This protocol describes the basic methods and recommendations for the proper handling and use of the TruVivo culture system for the evaluation of potential compound-induced changes in Cytochrome P450 (CYP) enzymes in primary human hepatocytes by quantitative polymerase chain reaction (qPCR).

Precautions

Observe universal precautions when handling human-derived tissues and cells as they are potentially biohazardous and wear appropriate PPE. Refer to the guidelines set forth in Occupational Safety and Health Standards for handling blood, tissues, body fluids, or other potentially infectious materials. Follow institutional guidelines for the collection and disposal of all solid and liquid waste that has been in contact with these products.

Methods

1. Start human TruVivo cultures, 24-well* format following the TruVivo Instructions for Use.
2. Maintain cultures with daily media changes, using TruVivo Culture Medium (TCCM), through the start of compound treatment. Typically, compound addition will begin on day 7 of culture (168 hours post-plating) to allow for sufficient acclimation of the model but can start as early as day 5 as needed.
3. Test article and reference compound (Table 1) stock solutions can be prepared, at 1000x the desired treatment solution, on the initial day of treatment or prior if stored properly.

   NOTE: Keep stocks sterile and use appropriate solvents to maximize substrate solubility while minimizing enzyme inhibition (e.g., DMSO, ACN, MeOH). Suggest the final solvent volume in the treatment solution does not exceed 0.1%.
4. On day 7, dilute the stock test article and reference compound solutions 1:1000 in warmed (37°C) TCCM.

   NOTE: Keep medium away from overhead or UV light and limit time in water bath to less than 60 minutes.
5. Remove TruVivo cultures from the CO_2 incubator and visually inspect the plates to ensure that the hepatocyte and feeder cell layers are intact and representative of the expected attachment, uniformity, and morphology for the respective cell lots being used.
6. In a biological safety cabinet and using sterile techniques, aspirate the medium from each well and replace with 0.5 mL of prepared treatment solution (suggest a minimum of three wells per treatment solution/concentration). Immediately following addition of the treatment solutions, return culture plates to the CO_2 incubator.
7. Repeat step 6 daily, including preparation and addition of fresh treatment solutions, every 24 hours (±30 minutes) through day 10.
8. On day 11, after a total compound incubation time of 96 hours, remove TruVivo cultures from the CO_2 incubator and place in a biological safety cabinet. Aspirate the medium from each well and rinse cultures with phosphate-buffered saline (PBS, 1X).
9. Add 350 µL of Buffer RLT (RNeasy Mini kit, Qiagen) containing β-mercaptoethanol to each well and place culture plate(s) at -80°C for 60 minutes.
10. Following the 60-minute period, remove culture plate(s) from the -80°C freezer and place on ice to thaw. Once samples are thawed, mix thoroughly and collect the cells. Isolate RNA from each sample with the RNeasy Mini kit according to the manufacturer's instructions.
11. Following quantification of each sample's RNA concentration, perform reverse transcription to synthesize cDNA.
12. Analyze the cDNA samples by qPCR using gene-specific TaqMan expression assays for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and/or CYP3A4 and an endogenous housekeeping gene GAPDH (glyceraldehyde 3-phosphate dehydrogenase). (See Ramsden et al., 2025 for suggestions or request further information.)
13. Following the comparative threshold cycle method ($\Delta\Delta C_T$; Applied Biosystems User Bulletin 2), determine the relative-fold mRNA content of the target gene relative to the endogenous control for each reaction and normalized to vehicle control.

Table 1. Positive Controls for CYP Induction.

Enzyme	Inducer	Stock Solution Concentration (mM)	Final Treatment Concentration (µM)
CYP1A2	Omeprazole	50	50
CYP2B6	CITCO	0.25	0.25
CYP2C8	Rifampicin	10	10
CYP2C9	Rifampicin	10	10
CYP2C19	Rifampicin	10	10
CYP3A4	Rifampicin	10	10

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Materials

1. Consumables
   - 70% (v/v) alcohol (ethanol or isopropyl alcohol)
   - 70% alcohol wipes (or lab wipes soaked in 70% (v/v) alcohol)
   - Laboratory wipes
   - Ice
   - Liquid nitrogen (LN_2)
   - Sterile 15 mL and 50 mL conical centrifuge tubes
   - Sterile 50 mL or 100 mL reagent reservoirs (optional)
   - Omeprazole (Sigma-Aldrich, Catalog #O104)
   - CITCO (Sigma-Aldrich, Catalog #C6240)
   - Rifampicin (Sigma-Aldrich, Catalog #R3501)
   - LifeNet Health Cryopreserved Feeder Cells
   - LifeNet Health Cryopreserved Human Hepatocytes
   - LifeNet Health Feeder Cell Thawing Medium (FCTM)
   - LifeNet Health TruVivo Plating Medium (TCPM)
   - LifeNet Health TruVivo Culture Medium (TCCM)
   - LifeNet Health TruVivo Supplement A (TCSA)
   - LifeNet Health TruVivo Supplement B (TCSB)
   - LifeNet Health TruVivo Supplement C (TCSC)
   - Antibiotic (optional; product suggestion – Penicillin/Streptomycin)
   - Trypan blue solution or Acridine orange/Propidium iodide (AO/PI) stain
   - Sterile serological pipettes
   - Sterile filtered tips for single and multi-channel pipettes
   - 24-well collagen-coated plates (BioCoat™; Corning, Catalog #354408)
   - Sterile media bottles
   - Sterile PES (polyethersulfone) filter units, 0.2 µm
   - Sterile plastic or glass aspiration tips
   - RNeasy Mini kit (Qiagen, Catalog #74104 or 74106)
2. Small Equipment
   - Hemocytometer or other cell-counting device (e.g., Revvity/Nexcelom Cellometer Spectrum cell counter)
   - Single and multi-channel pipettes
   - Serological pipettor (e.g., Pipet-Aid)
   - Forceps or tongs
   - Laboratory ice tray capable of containing a small amount of LN_2 and a cryovial box
   - Timer
   - NanoDrop™ Spectrophotometer
   - SimpliAmp™ Thermal Cycler (or equivalent)
3. Large Equipment
   - Cryogenic storage freezer capable of temperatures ≤-150°C
   - 4°C refrigerator
   - -20°C freezer
   - -80°C freezer
   - Biological safety cabinet
   - Portable liquid nitrogen (LN_2) dewar or other suitable container to transport frozen vials
   - Vacuum aspiration system with waste flask
   - Centrifuge capable of achieving up to 400 x g (50 mL conical tube adaptors)
   - 37°C water bath (operation range of 35-38°C)
   - CO_2 incubator, humidified (settings of 36.5-37.5°C and 4.5-5.5% CO_2)
   - Inverted microscope with brightfield optics or phase contrast
   - Analytical balance
   - Vortex mixer/shaker
   - Microcentrifuge capable of achieving up to 12,000 x g
   - QuantStudio™ 6 Pro Real-Time PCR System (or equivalent)

References

Ramsden D, Fullenwider CL, Santos C, and LeCluyse EL (2025) Quantitative clinical risk assessment of CYP2C, UDP-glucuronosyltransferase, P-glycoprotein induction, and complex drug-drug interactions using TruVivo human hepatocyte triculture platform. *Drug Metab Dispos* **53**: 100052.

*This protocol is designed for 24-well format. If you need instructions specific for 96-well format, please contact your LifeNet Health LifeSciences Sales representative or email lifesciences@lifenethealth.org for technical support.

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