



*~ Assay Kit ~*

**Expression Profiling of Clinically Relevant CYPs  
Utilizing upcyte® Hepatocytes**

(CYP3A4, CYP1A1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1)

**2x 48 Assays in 96-well Format**  
Product # UGE1003-48

■

**Technical Manual**  
*(version 1)*

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## I. Description

### ▪ Background ▪

#### **Human upcyte<sup>®</sup> Hepatocytes**

Historically, primary hepatocytes have been the preferred *in vitro* model for assessing drug-induced expression of drug metabolizing enzymes. However, their limited supply from any one donor and their finite life span pose challenges to their routine use for iterative, comparative drug screening.

Immortalized human hepatoma (*i.e.*, HepG2) and hepato-carcinoma cell lines are sometimes used owing to their unlimited proliferative potential. However, a serious limitation to using transformed cell lines is their decreased, or absent, expression of hepatocyte differentiation markers. These include nuclear receptors and other xenobiotic-sensing receptors and, hence, their target genes (*e.g.*, CYPs) whose expression are critical to correctly assessing the potential liability of induced drug-drug interactions.

This kit utilizes **upcyte<sup>®</sup> hepatocytes**, which are human donor-derived hepatocytes established by upcyte<sup>®</sup> technologies (GmbH)<sup>1-3</sup>. These cells have the attribute of limited proliferation while maintaining their native levels of constitutive and inducible xenobiotic metabolizing enzyme activities. Like primary hepatocytes, confluent cultures of upcyte<sup>®</sup> hepatocytes express liver-specific proteins, produce urea and store glycogen. Importantly, the induction profiles of cytochrome P450 (CYP) enzyme activities are similar to those of primary hepatocytes. Thus, upcyte<sup>®</sup> hepatocytes combine the characteristics and advantages of primary hepatocytes with the added practical advantage of having access to the same donor cells for use in iterative, large-scale experiments over extended periods.

#### **Clinically Relevant CYP Enzymes**

Cytochrome p450 (CYP) enzymes are responsible for the Phase I metabolism of most drugs. And, it is noteworthy that the expression of cytochrome P450 (CYP) genes are predominantly regulated by ligand-activated receptors/transcription factors such as pregnane X receptor (PXR, NR1I2), constitutive androstane receptor (CAR, NR1I3), aryl hydrocarbon receptor (AhR), farnesoid X receptor (FXR, NR1H4), glucocorticoid receptor (GR, NR3C1), and to lesser degrees liver X receptors (LXR, NR1H3), vitamin D receptor (VDR, NR1I1), and peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ , NR1C1)<sup>4</sup>. Consequently, drugs that activate any of these xenobiotic sensing receptors can dramatically change the endogenous levels of CYP expression in the liver, potentially impacting the rate of their own metabolism, as well as the metabolism of all other co-administered drugs. Of particular concern are metabolic outcomes that transform a drug to greater potency, or to an altered bioactivity.

Assessing drug-induced changes in the expression of CYP genes provides a reliable predictive indicator of altered (either heightened or inhibited) metabolic activities leading to drug-drug interactions *in vivo*. Cytochrome P450 enzymes with the greatest clinical relevance belong to CYP1, 2 and 3 families. It is estimated that they are involved in the metabolism of 70-80% of drugs currently on the market<sup>4</sup>. For example, CYP3A4 is estimated to be involved in the metabolic conversion of ~ 30% of all prescription drugs.

This assay kit provides optimized reagents for the culturing and treatment of upcyte<sup>®</sup> hepatocytes to assess drug-induced changes in the expression of seven clinically relevant CYPs<sup>5</sup>: **CYP3A4, CYP1A1, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2E1**.

▪ **The Assay System** ▪

The reagents and materials provided in this assay kit for the **Expression Profiling of Clinically Relevant CYPs** are formatted to allow for two alternative cell culture setups. In one scenario 48 culture wells may be setup at two different times. In the other assay scenario 96 culture wells may be setup at one time. The kit includes two aliquots of upcyte® hepatocytes, donor 10-03, isolated from an adult Caucasian female<sup>1-3</sup>. These hepatocytes are cryopreserved using INDIGO's proprietary **CryoMite™** process, which yields high cell viability post-thaw, and provides the convenience of immediately dispensing cells into assay plates. There is no need for intermediate treatment steps such as spin-and-rinse of cells, viability determinations, or cell titer adjustments prior to assay setup. During an overnight culture period for cell recovery the hepatocytes will form a confluent monolayer that is ready to receive treatment media containing the user's test compounds.

In addition to two aliquots of upcyte® hepatocytes, the kit provides two cell culture-ready assay plates, optimized Cell Culture Medium (CCM) for use in all steps of the assay procedure (cell thawing, seeding, and preparation of treatment media), three reference compounds (rifampicin,  $\beta$ -naphthoflavone, and chenodeoxycholic acid (CDCA)) that activate one or more of the primary xenobiotic-sensing receptors PXR, CAR, AhR and FXR. Upon activation, these nuclear receptors modulate the expression of the clinically relevant CYP genes. Finally, as described in **Table 1**, included are seven sets of validated qPCR primers for quantifying drug-induced changes in the expression of CYP3A4, CYP1A1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2E1, as well as primers for ACTB ( $\beta$ -actin; the internal control used to normalize all CYP gene expression data).

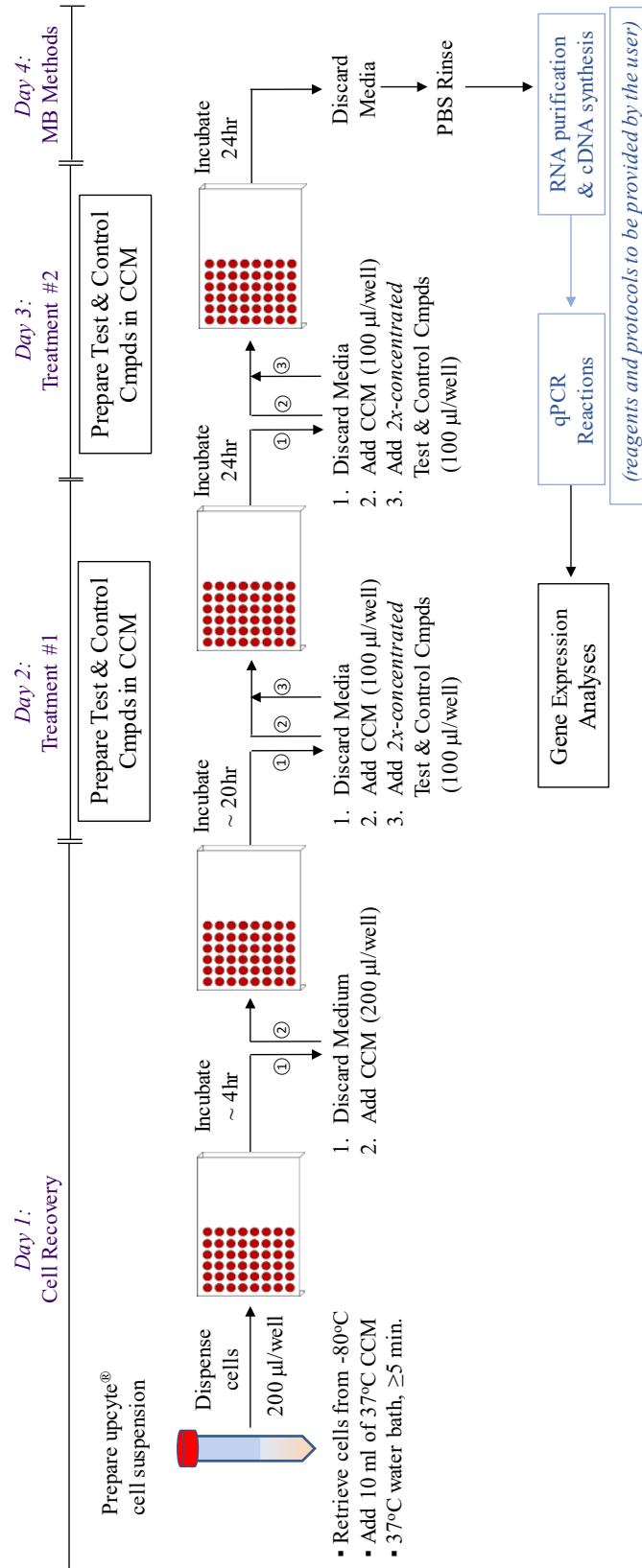
**Please NOTE: This kit does not include reagents or protocols for cell lysis, RNA isolation, cDNA preparation or qPCR assays.**

**Table 1.** Primer sequences and accession numbers of the target genes. The primers provided in this kit are intended for qPCR chemistries utilizing fluorogenic intercalating dyes, such as SYBR green.

<b>CYP Target Gene</b> (accession no.)	<b>Forward Primer</b> Sequence (5'-3')	<b>Reverse Primer</b> Sequence (5'-3')
<b>CYP3A4</b> (NM_017460.5; 1 other variant)	GTGGTGATGATTC CAAGCTATGC	TCCTTGTTCTTCTTG CTGAATC
<b>CYP1A1</b> (NM_000499.4; 2 other variants)	GATTGAGCACTGT CAGGAGAAGC	ATGAGGCTCCAGGA GATAGCAG
<b>CYP2B6</b> (NM_000767.4)	ACAGTGTGGAGAA GCACCGTGA	GGTTGAGGTTCTGG TGGCTGAA
<b>CYP2C8</b> (NM_000770.3; 3 other variants)	GAGACAACAAGCA CCACTCTGAG	CAGTGTAAGGCATG TGGCTCCT
<b>CYP2C9</b> (NM_000771.3)	CAGAGACGACAAG CACAACCCT	ATGTGGCTCCTGTCT TGCATGC
<b>CYP2C19</b> (NM_000769.3)	CTGGCTGAAAGAG CTAACAGAGG	TGAACACGGTCCTC AATGCTCC
<b>CYP2E1</b> (NM_000773.3)	GAGCACCATCAAT CTCTGGACC	CACGGTGATACCGT CCATTGTG
<b>ACTB</b> (NM_001101.4)	CACCATTGGCAAT GAGCGGTTC	AGGTCTTTGCGGAT GTCCACGT

▪ Assay Scheme ▪

**Figure 1.** Assay workflow for 48hr drug treatment of upcyte® Hepatocytes.



#### ▪ Preparation of Test Compounds, Reference Compounds, Vehicle Control Media ▪

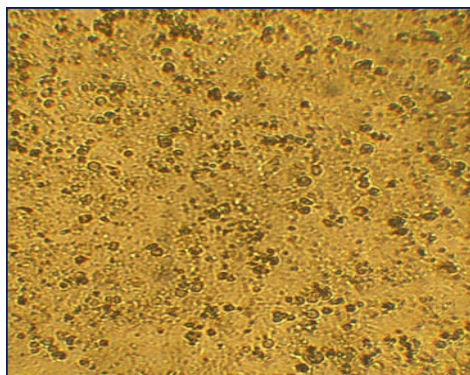
Test and Reference compounds are typically solvated at high-concentration in DMSO and stored frozen as master stocks. To help avert insolubility of compounds at high treatment concentrations, and to limit the amount of DMSO carried over into the culture wells, it is recommended that master stocks are diluted using DMSO to generate a series of 1,000x-concentrated stocks relative to *each* treatment concentration. In brief, DMSO is used to make serial dilutions of master stocks to generate the desired sub-concentrations that are also 1,000x-concentrated. Cell Culture Medium (CCM) is then used as the final diluent to generate 500-fold dilutions of each DMSO intermediate stock, thereby generating 2x-concentrated treatment media. At *Step 14* of the assay protocol 100  $\mu$ L of the 2x-concentrated treatment media will be dispensed into respective culture wells containing upcyte<sup>®</sup> hepatocytes in 100  $\mu$ L of CCM, thus achieving the desired 1x drug concentrations in each culture well. This dilution strategy limits the final concentration of DMSO to 0.1% in each culture well.

If an alternative dilution method is preferred, take care to *not* exceed 0.3% DMSO carried-over into the culture wells, as this concentration will degrade assay performance.

The series of 1,000x-concentrated stocks prepared in DMSO, and used on Day 2, may be stored at -20°C for later use on Day 3 to prepare the second series of 2x-concentrated treatment media. 2x-treatment media should always be prepared fresh!

Prepare CCM containing 0.2% DMSO only (*i.e.*, 2x-concentrated “vehicle only” control media). All drug-induced changes in CYP gene expression will be determined by comparing to their basal levels of expression in the “vehicle only” treated hepatocytes.

#### ▪ Assay Performance ▪



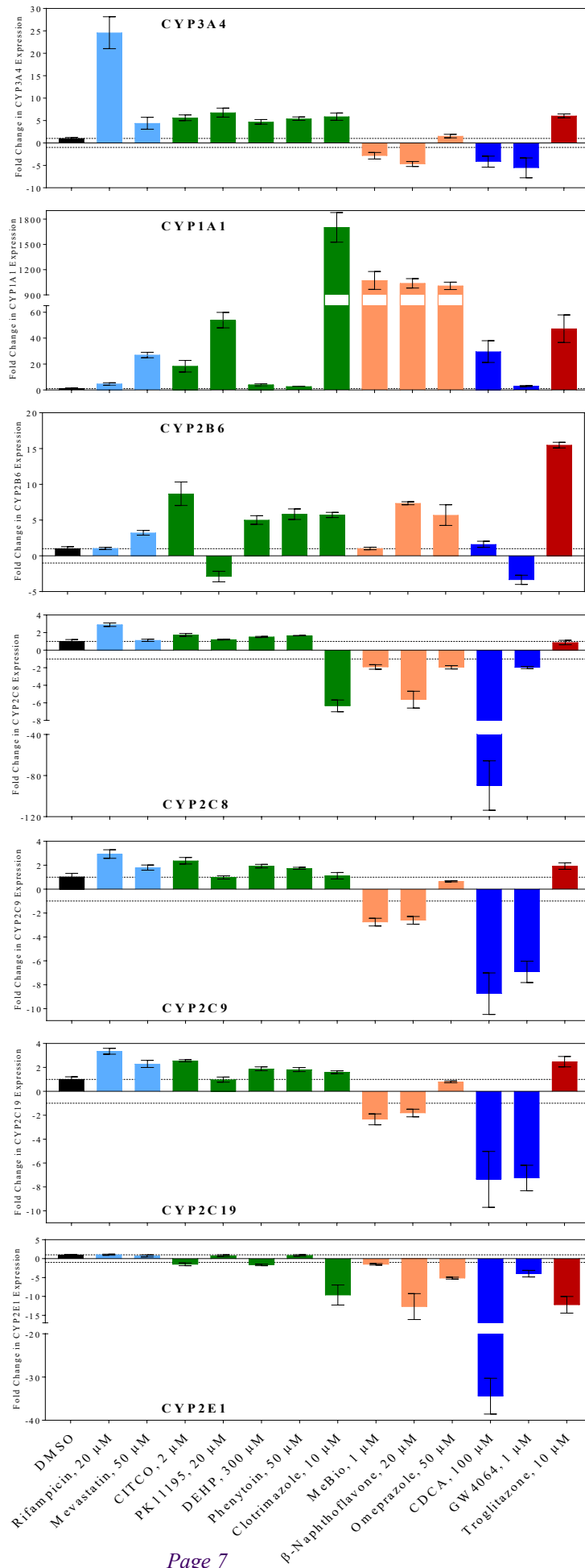
**Figure 2. Micrograph of Human upcyte<sup>®</sup> Hepatocytes Donor 10-03.**

Cells were thawed and treated as described in this manual. Representative micrograph taken at 40X, 48hr after treatment with either vehicle or reference compounds.

**Figure 3 (next page). Changes in gene expression patterns of clinically relevant CYPs in upcyte<sup>®</sup> Hepatocytes treated for 48hr with modulators of relevant nuclear receptors.**

upcyte<sup>®</sup> Hepatocytes were treated with prototypical ligands of the xenobiotic-sensing nuclear receptors PXR, CAR, AhR, FXR, and PPAR. Treatment concentrations, noted in the graph, were determined to be non-toxic to the hepatocytes. Rifampicin,  $\beta$ -naphthoflavone, and CDCA are inducers of PXR, AhR and FXR respectively, and they are provided in this kit as reference modulators of CYP gene expression. Following sequential 24hr + 24hr treatment periods, cell lysates were prepared and total RNAs were purified using Promega's SV Total RNA Isolation System reagents and protocol. Purified RNAs were converted into cDNAs using Applied Biosystems High Capacity cDNA reverse Transcription kit reagents and protocol. Quantitative PCR (qPCR) was performed using SYBR green reaction chemistry and validated primer sets for CYP3A4, CYP1A1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1 and ACTB (Table 1). These eight primer sets are provided in this assay kit as 20x concentrated stocks. All primers were used at a final concentration of 100 nM, except for CYP2E1 primers which were used at 400 nM. Drug-induced changes in the expression of the CYPs were quantified using the  $\Delta\Delta C_t$  analytical method; the expression of ACTB was used as the internal reference for normalizing qPCR results between sample replicates. Depicted are fold-changes in CYP gene expression relative to DMSO (0.1% v/v) treated controls.

**Figure 3**



## II. Product Components & Storage Conditions

This assay kit contains 2 aliquots of frozen upcyte<sup>®</sup> hepatocytes to allow for two separate cell culture setups, each comprising 48 wells. Alternatively, cell suspensions generated from the two individual aliquots may be combined for one 96 well assay setup. Two 96-well collagen-coated tissue culture plates are provided to accommodate the user's preferred assay format. If performing a 48 well cell culture setup, *refreeze the extra volumes of all reagents*.

Assay kits are shipped on dry ice. Upon receipt, individual kit components may be stored at the temperatures indicated on their respective labels. More convenient, however, is to keep kit components together and simply store the entire assay kit in a -80°C freezer.

To ensure maximal viability, upcyte<sup>®</sup> hepatocytes must be continuously maintained at no warmer than -78°C until immediately prior to use. Do not store cells in liquid nitrogen.

The date of product expiration is printed on the Product Qualification Insert (PQI) enclosed with each kit

<u>Kit Components</u>	<u>Amount</u>	<u>Storage Temp.</u>
▪ upcyte <sup>®</sup> Hepatocytes	2 x 1.0 mL	-80°C
▪ Cell Culture Medium (CCM)	1 x 150 mL	-20°C
▪ Rifampicin, 1,000x (20 mM in DMSO; positive control for modulators of CYP3A4 and CYP2C genes)	1 x 30 µL	-80°C
▪ β-Naphthoflavone, 1,000x (20 mM in DMSO; positive control for modulators of CYP1 and CYP2 genes)	1 x 30 µL	-80°C
▪ CDCA, 1,000x (100 mM in DMSO; positive control for modulators of CYP1A1, CYP2C, CYP2E1, and CYP3A4 genes)	1 x 30 µL	-80°C
▪ 8 Primer sets (all prepared in water at 20x concentrations)		
CYP3A4, 2 µM	1x 65 µL	-20°C
CYP1A1, 2 µM	1x 65 µL	-20°C
CYP2B6, 2 µM	1x 65 µL	-20°C
CYP2C8, 2 µM	1x 65 µL	-20°C
CYP2C9, 2 µM	1x 65 µL	-20°C
CYP2C19, 2 µM	1x 65 µL	-20°C
CYP2E1, 8 µM	1x 65 µL	-20°C
β-Actin (ACTB), 2 µM	1 x 130 µL	-20°C
▪ 96-well cell culture-ready assay plate (sterile, collagen-coated)	2 plates	-20°C

**NOTE 1:** The primer sets provided in this kit are a premix of unlabeled PCR forward and reverse primers that were validated using SYBR Green qPCR chemistry. These primers are not compatible with TaqMan qPCR chemistry.

**NOTE 2:** This kit contains two 96-well tissue culture plates in which the wells have been collagen-coated and dried; the culture plates should be stored frozen (-20°C or colder) until use.



### ***III. Materials to be Supplied by the User***

The materials listed below must be provided by the user and should be made ready for use prior to commencing the assay procedure.

#### ***Additional Commercial Kits & Reagents Required:***

- Total RNA Purification kit (containing cell lysis reagent) of user's choice. *Note:* It is necessary to have 'cell lysis reagent for RNA purification' available for immediate use on DAY 4 of the assay protocol.
- Reverse Transcription kit of user's choice
- Quantitative PCR kit using SYBR Green chemistry, of user's choice

#### ***DAYS 1, 2, & 3: Cell Recovery and Cell Treatments***

- dry ice for Protocol *Step 3*
- Cell culture-rated laminar flow hood
- Cell culture incubator (37°C, ≥85% humidity, 5% CO<sub>2</sub>)
- 37°C water bath
- 70% alcohol wipes
- 8-channel electronic pipettes capable of repeat-dispensing 100 µl and 200 µl volumes, and sterile tips
- 8-channel manual pipettes (0.5-10 µl) & sterile tips
- Disposable media basins, sterile
- Dimethyl sulfoxide (DMSO)
- Sterile 96-well PCR plates to prepare 1,000x stocks of reference compound(s) and test compound(s)
- Sterile multi-channel media basins (such as the Heathrow Scientific "Dual-Function Solution Basin"), *or* deep-well plates, *or* appropriate similar vessel for generating 2x-concentrated dilution series of reference compound(s) and test compound(s)
- Waste container for 'media-dump' steps, and clean absorbent paper towels

#### ***DAY 4: Cell Harvest***

- 8-channel electronic pipettes capable of repeat-dispensing 100 µl and 200 µl volumes, and sterile tips
- Disposable media basins, sterile
- Waste container for 'media-dump' steps, and clean absorbent paper towels
- Phosphate Buffered Saline (PBS)

#### IV. Assay Protocol

Before starting, carefully review the entire assay protocol, as well as the list of “*Materials to be Supplied by the User*” (pg. 9).

The Day 4 protocol requires the use of two critical reagents that must be provided by the user: PBS (*Step 23*) and cell culture lysis reagent for RNA purification applications (*Step 25*).

**DAY 1 Cell Recovery:** All steps must be performed using aseptic techniques.

**1.) Remove Cell Culture Medium (CCM)** from freezer storage, thaw and equilibrate to 37°C using a water bath. Mix the solution by inverting the bottle several times.

**2.) Remove a culture-ready plate** from -20°C and place in a 37°C incubator to allow temperature equilibration. If intending to treat 48 wells of upcyte® Hepatocytes, then only half of one 96-well culture plate will be utilized.

**3.) Rapid Thaw of upcyte® Hepatocytes:** *First*, retrieve the bottle of CCM from the 37°C water bath and sanitize its outside surfaces with a 70% ethanol swab.

*Second*, if intending to setup 48 treatment wells retrieve one tube of upcyte® Hepatocytes from -80°C storage and *immerse the tube in dry ice* to transport it to a laminar flow hood. When ready to proceed, place the tube of cells in a rack and, *without delay*, perform a rapid thaw of the frozen cells by transferring 10 ml from the bottle of pre-warmed CCM into the tube of frozen cells. Recap the tube and immediately place it in a 37°C water bath for a minimum of 5 minutes. The resulting volume of cell suspension will be 11 ml.

*NOTE:* If intending to setup 96 treatment wells then perform a rapid thaw of each tube of upcyte® Hepatocytes. In *Step 5* the two cell suspensions will be combined in a sterile media basin to produce a total volume of 22 ml.

**4.)** Retrieve the tube(s) of upcyte® Hepatocytes from the water bath and sanitize the outside surface with a 70% alcohol swab.

**5.)** *Gently* invert the tube(s) of upcyte® Hepatocytes several times to disperse cell aggregates and gain a homogenous cell suspension. Caution: do *not* mix the cell suspension by pipetting up and down. Transfer the cell suspension into a sterile media basin. Using a multichannel repeat-dispensing pipette, dispense **200 µl / well** of the cell suspension into the culture plate.

*NOTE:* Increased well-to-well variation will occur if care is not taken to prevent cells from settling during the dispensing period. Likewise, take care to dispense uniform volumes across the assay plate.

**6.) Post-thaw recovery of upcyte® Hepatocytes:** Incubate the assay plate for ~4hr in a cell culture incubator (37°C, ≥ 85% humidity, 5% CO<sub>2</sub>).

*NOTE:* Ensure a ≥ 85% humidity environment within the cell culture incubator. This is critical to prevent the onset of deleterious "edge-effects" in the assay plate.

***Near the end of the 4-hour recovery period:***

**7.)** Retrieve the bottle of pre-warmed (37°C) CCM from the water bath and sanitize the outside surface with ethanol wipes.

**8.)** At the end of the ~4hr cell recovery period, **discard the culture medium** by ejecting it into an appropriate waste container. *Gently* tap the inverted plate onto a clean absorbent paper towel to remove residual droplets.

**9.)** Dispense **200 µl** of CCM into each well and incubate the assay plate for an additional ~20hr in a cell culture incubator.

**DAY 2, First Treatment:** All steps must be performed using aseptic techniques.

*Near the end of the 20hr incubation period:*

10.) Retrieve the bottle of CCM from refrigerator storage and allow it to equilibrate to room temperature.

11.) Prepare Test and Reference Compounds in CCM to generate '2x-concentrated' treatment media. Manage dilution volumes carefully; this assay kit provides **150 ml** of CCM.

As discussed in "Preparation of Test Compounds" (pg. 6), it is recommended that master stocks are serially diluted in DMSO to generate intermediate stocks at '1,000x-concentration' relative to *each* final treatment concentration. These 1,000x stocks are then diluted 500-fold using CCM, thereby producing the '2x-concentration' treatment media that will be dispensed into respective culture wells (*Step 14*).

Positive Control treatments that elicit changes in CYP gene expression (as depicted in **Figure 3**) are 20  $\mu$ M Rifampicin (CYP3A4, CYP2C), 20  $\mu$ M  $\beta$ -Naphthoflavone (CYP1A1, CYP2B6), or 100  $\mu$ M CDCA (CYP2E1, CYP2C19, CYP2C9, CYP2C8). These three reference compounds are provided in this kit as 1,000x concentrated stocks prepared in DMSO. As with the user's test compounds, use CCM to make 500-fold dilutions of the desired reference solution(s) to generate respective '2x-concentrated' Control treatment media.

*NOTE:* This recommended strategy for preparing treatment media results in 0.1% DMSO carried over into the culture wells. If an alternative strategy is preferred for making the various treatment media, it is important that the total DMSO carried over into culture wells does **not exceed 0.3%**.

12.) At the end of the ~20hr pre-treatment culture period, **discard the culture media** by ejecting it into an appropriate waste container. *Gently* tap the inverted plate onto a clean absorbent paper towel to remove residual droplets.

13.) Dispense **100  $\mu$ l** of CCM into appropriate culture wells.

14.) Dispense into respective culture wells **100  $\mu$ l** of each prepared 2x-concentrated treatment media (Vehicle only, Nuclear receptor Reference Ligands, and Test Compounds).

15.) Incubate the cells for 24hr in a cell culture incubator.

**DAY 3, Second Treatment:** All steps must be performed using aseptic techniques.

*Near the end of the 24-hour treatment period:*

16.) Remove CCM from refrigerator storage and equilibrate to 37°C in a water bath.

17.) Use CCM to prepare fresh **Test Compound** and **Reference Compound treatment media**, as was done in *Step 11* (on Day 2).

18.) At the end of the 24hr incubation period, **discard the culture media** by ejecting it into an appropriate waste container. *Gently* tap the inverted plate onto a clean absorbent paper towel to remove residual droplets.

19.) Dispense **100  $\mu$ l** of CCM into appropriate assay wells.

20.) Dispense into respective culture wells **100  $\mu$ l** of each prepared 2x-concentrated Reference and Test Compound(s) treatment media.

21.) Incubate the cells for an additional **24hr** in a cell culture incubator.

**DAY 4, Molecular Biology Methods:** Subsequent manipulations do *not* require special regard for aseptic techniques and may be performed on a bench top.

**22.)** At the end of the second 24-hour treatment period, discard the culture media by ejecting it into an appropriate waste container. *Gently* tap the inverted plate onto a clean absorbent paper towel to remove residual droplets. Cells will remain tightly adhered to well bottoms.

**23.) Rinse:** Dispense into each well **200 µl** of room temperature Phosphate Buffered Saline (PBS; provided by the user). Manually rinse the cells by gently rocking the plate 2-3 times.

**24.)** Discard the PBS by ejecting it into an appropriate waste container. *Gently* tap the inverted plate onto a clean absorbent paper towel to remove residual droplets.

**25.)** Add your preferred **cell lysis reagents for RNA purification** to each culture well, as per the recommendation of the manufacturer.

*NOTE:* At this point, depending on the specific cell lysis reagent and RNA isolation method used, the user may be able to store the lysates at **-80°C** and continue with RNA isolation at a later time; consult the protocol recommendation of the manufacturer.

**26.)** Using your preferred assay kit reagents and protocols proceed with RNA isolation, cDNA synthesis and qPCR reaction setup. See “*Section V. Recommendations for cDNA and qPCR preparations*” for comments and general suggestions.

## V. Recommendations for cDNA and qPCR preparations

The CYP gene expression data presented in this Technical Manual were generated using the following commercial kit reagents and protocols:

- Promega SV96 total RNA isolation vacuum system for high throughput RNA isolation
- Applied Biosystems high capacity cDNA kit for cDNA preparation
- Quanta Biosciences PerfeCta SYBR Green Fast Mix® ROX and ABI Step One Plus Real Time PCR Fast 2-Step cycling system.

In general, ~200-300 ng of total RNA was reverse transcribed into cDNA. The cDNA preparation was diluted 30-fold with water, and 2.5 µl of the diluted cDNA template was used in the setup of a 10 µl qPCR reaction, as described in **Table 2**. PCR reactions were performed over 40 cycles, at an annealing/extension temperature of 58°C over 30 sec. Alternative RNA isolation methods, cDNA synthesis reagents, and SYBR Green qPCR kit reagents are expected to yield comparable results.

**Table 2.** Example preparation of qPCR reaction mixes, 10 µl per single reaction.

qPCR Reagents	Mix for 1 qPCR Rxn	~ 8% excess volume / mix	
		Master Mix 48 qPCR Rxns	Master Mix 96 qPCR Rxns
2x Master Mix Syber Green Rgt.	5.0 µl	260 µl	520 µl
CYP or ACTB Primer Mix, 20x-conc.	0.5 µl	26 µl	52 µl
Water (nuclease-free)	2.0 µl	104 µl	208 µl
Mix Volume	7.5 µl	390 µl	780 µl
<div>↓ ↓ ↓</div>			
PCR Plate	Dispense 7.5 µl per well		
<div>↑ ↑ ↑</div>			
cDNA rxn mix (diluted with water)	2.5 µl	2.5 µl / rxn	2.5 µl / rxn

## VI. Related Products

Validated Primer Sets for qPCR	
<i>Product No.</i>	<i>Product Descriptions</i>
CYP3A4-65	CYP3A4 qPCR primer mix, 20x-concentrated, 65 µl
CYP1A1-65	CYP1A1 qPCR primer mix, 20x-concentrated, 65 µl
CYP2B6-65	CYP2B6 qPCR primer mix, 20x-concentrated, 65 µl
CYP2C8-65	CYP2C8 qPCR primer mix, 20x-concentrated, 65 µl
CYP2C9-65	CYP2C9 qPCR primer mix, 20x-concentrated, 65 µl
CYP2C19-65	CYP2C19 qPCR primer mix, 20x-concentrated, 65 µl
CYP2E1-65	CYP2E1 qPCR primer mix, 20x-concentrated, 65 µl
ACTB-130	β-Actin qPCR primer mix, 20x-concentrated, 130 µl

## ***VII. Limited Use Disclosures***

upcyte® Hepatocytes are used by INDIGO Biosciences through commercial license agreement with upcyte technologies, GmbH (Hamburg, Germany).

Products commercialized by INDIGO Biosciences, Inc. are for RESEARCH PURPOSES ONLY – not for therapeutic, diagnostic, or any form of contact application use in humans or animals.

“CryoMite” is a Trademark <sup>TM</sup> of INDIGO Biosciences, Inc. (State College, PA, USA)

Product prices, availability, specifications and claims are subject to change without prior notice.

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