HμREL CORPORATION

HμRELflux™

Biliary Efflux Assay Kit

Frequently Asked Questions (FAQ's)

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1) What is HμRELflux™?

HμRELflux™ is a novel, patent-pending method for assessing a compound's biliary disposition.

2) Why is biliary disposition important?

Transporters can play an important role in the clearance and excretion of the parent drug and/or its metabolite(s) into the bile canaliculus. In-vitro methods for accurately predicting such dispositions are of great need.

3) What data does the HμRELflux™ assay return to the user?

With this assay one can measure:

a) Compound clearance from the dosing solution
b) Biliary accumulation
c) Intracellular accumulation

With these data one can predict a compound's:

a) Intrinsic clearance
b) Uptake rate
c) Biliary clearance
d) Biliary excretion index

4) Does HμRELflux™ use gel overlays?

No. Gels can be cumbersome to apply and may cause non-specific binding of your compounds. HμREL® primary hepatic co-cultures readily form bile canaliculi, there is no need to use special "Certified" cells

5) How does the HμREL® model achieve the levels of hepatic function and morphology necessary to form bile canaliculi?

HμREL® uses its own patented primary hepatocyte co-cultures which allow the hepatocytes to polarize and form stable and enduring biliary canaliculi networks and their associated transporters for over 30 days.

6) How does HμREL® measure the relatively small quantities of bile in the cultures?
In order to accurately quantify the biliary clearance of a compound we:
a) Take all of our measurements in one well and assess the biliary portion and cellular portion from the same single well.
b) Take accurate measurement of small quantities by LC/MS.

7) Do the compounds being assessed need to be radio labeled?
No, radio or fluorescent labeling is not required. LC/MS can provide accurate measurement without need to label compounds.

8) Is it possible to generate a negative biliary excretion index ("BEI")?
No. Because HμREL*flux*™ takes direct measurement of both the biliary and, separately, the intracellular portions—all from within a single well of hepatocytes, with no arithmetic comparison of results obtained from multiple wells—it is impossible to generate a negative BEI number.

9) How does HμREL® account for basolateral transport during the canalicular disruption phase of the experiment?
While it is certainly possible for a compound to be efluxed via basolateral transporters in vitro; we believe that this parameter is negligible for most compounds and have engineered our assay to minimize the effect that this phenomena may have on your biliary excretion readings.

10) Is HμREL*flux*™ available as a kit?
Yes, The HμREL*flux*™ biliary efflux assay kit Is air-shipped by overnight courier to your lab, where it arrives ready for immediate, "plug-and-play" use after a brief period of a few hours' acclimation. No plating and only minimal tissue culture is required of the customer.

11) What does the HμREL*flux*™ biliary efflux assay kit contain?
Media (both maintenance and dosing media), HμREL® primary hepatocyte co-cultures, all reagents necessary to perform the HμREL*flux*™ assay, detailed protocol, unpacking protocol and certificate of analysis will be included in the HμREL*flux*™ kit.

12) Is HμREL*flux*™ also available on a contract research services basis?
Yes. HμREL® expert technical staff will perform the HμREL*flux*™ assay on your compound(s) if you prefer to outsource this workflow. Please contact HμREL® for more information on HμREL*flux*™ contract research services.

13) Liver models of which species have been characterized for HμREL*flux*™?
HμREL® primary hepatic co-cultures are available in a variety of species. Currently, the HμREL*human*™, HμREL*primate*™, HμREL*dog*™, and HμREL*rat*™ patented models have been qualified using the HμREL*flux*™ method.

14) Which uptake transporters have been assessed using the HμREL*flux*™ method?

Probe substrates	Uptake transporters
Taurocholic acid (2μM)	NTCP, OATPs
Rosuvastatin (2μM)	OATPs
Digoxin (2μM)	OATP-8
Estradiol-17b-D-glucuronide (2μM)	OATPs
Pravastatin (5 μM)	OATPs

15) Which efflux transporters have been assessed using the HμREL*flux*™ method?

Probe substrates	Uptake transporters
Taurocholic acid (2μM)	BSEP
Rosuvastatin (2μM)	BCRP/MRP2
Digoxin (2μM)	P-gp, mdrp-3
Estradiol-17b-D-glucuronide (2μM)	MRP2
Pravastatin	BCRP/MRP2

16) How long should you wait after receiving the cells before beginning your experiment?
You can start your experiments using HμREL®'s primary hepatic co-cultures after a minimum four-hour re-acclimation period in an incubator, after replacement of the shipping media with the supplied maintenance media that is provided as a component of the kit. Alternatively, you can wait as long as three days after receiving your HμREL® co-cultures before beginning your experiments, with no diminution of hepatic function.

17) How long will an experiment take using the HμREL*flux*™ method?

Our customers have conducted their experiments over various time courses. Given the robustness of the HμREL® system, one has the ability to evaluate a variety of different rates, from a minimum of one minute to a maximum of four days. The HμREL*flux*™ method allows for easy biliary uptake/efflux evaluation, even of compounds with properties that make evaluation difficult when using traditional transporter evaluation methods.

18) Have in vitro-to-in vivo ("IVIVC") correlations been made for results obtained using HμREL*flux*™?

Yes. Using the HμREL*rat*™ hepatic co-culture model, in vitro-to-in vivo correlation was within 3-fold for all instances.

For more information:

Email the company at service@hurelcorp.com

Or call: (732) 253-0295