Suspension Metabolism of Cryopreserved Enterocytes

Enterocyte Reagents and Materials	**Order Information**	**Cat #**
IVAL Cryopreserved Suspension Enterocytes	IVAL	see PCS
Suspension Medium		
• HQM™ - Hepatocyte/Enterocyte Incubation Medium, 50 mL / 500 mL	IVAL	81039/81040

Laboratory Tools for Suspension Metabolism of Enterocytes

Prior to beginning, ensure the bench or Biological Safety Cabinet (BSC) is equipped with the following:

- 4°C HQM™
- appropriate size tubes for preparation of solutions
- pipettes and appropriate sterile tips
- 96 well non-coated tissue culture plate
- ice bucket containing ice
- isoform-selective substrates prepared at a 2x concentration in 37°C HQM™
    - 50 µM diclofenac (2C9)
    - 500 µM *S*-Mephenytoin (2C19)
    - 40 µM midazolam (3A4/5)
    - 200 µM 7-hydroxycoumarin (UGT, sulfotransferase)
    - 100 µM astemizole (2J2)
    - 100 µM irinotecan (CES2)

Prior to the end of the 2 hour incubation, ensure the bench or BSC is equipped with the following:

- acetonitrile prepared with 10 nM terfenadine and 50 nM tolbutamide at 4°C
- ice bucket containing ice

Suspension Metabolism Procedure

1. Place the tube of HQM™ in the ice bucket containing ice.
2. Prepare substrates at the 2x concentration in 37°C HQM™ and maintain in a 37°C waterbath.
3. Label the non-coated 96 well plate with the substrates that will be used in the experiment in duplicate.
4. Follow the IVAL protocol for thawing, counting, and re-suspension of the enterocytes and adjust the cell density to 3 million cells per mL. Maintain enterocytes on ice until it is time to aliquot the cells onto the 96-well plate.
5. Ensure the enterocytes are completely re-suspended. Add 50 µl to 2 wells per substrate (150,000 enterocytes/well).
6. After the cells are aliquoted, pre-incubate the plate in a 37°C incubator in 5% CO2/95% balanced air atmosphere for 15 minutes to bring the enterocytes to 37°C.
7. Following pre-incubation, initiate the reaction by adding 50 µl of the substrates prepared in 37°C HQM™ at the 2x concentration. The final concentration of the isoforms are as follows; 25 µM diclofenac, 250 µM *S*-mephenytoin, 20 µM midazolam, 100 µM 7-hydroxycoumarin,50 µM astemizole, and 50 µM irinotecan.
8. Incubate enterocytes with isoform specific substrates for 2 hours in a 37°C incubator.
9. To stop the reaction, remove the plate from the incubator and set on the bucket of ice. Add 100 µl of acetonitrile containing the internal standard to each well.
10. Seal the plate and store the samples at -80°C for LC-MS analysis for the marker metabolite.

Lot Specific Information

To inquire about our products and services or for technical questions please contact:

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