Thawing, Counting, and Re-suspension of Cryopreserved Enterocytes

Enterocyte Reagents and Materials	Order Information	Cat #
IVAL Cryopreserved Suspension Enterocytes	IVAL	see PCS
Thawing Medium		
• CERM™ - Cryopreserved Enterocyte Recovery Medium, 50 mL	IVAL	81056
Suspension Medium		
• HQM™ - Hepatocyte/Enterocyte Incubation Medium, 50 mL / 500 mL	IVAL	81039/81040

Laboratory Tools for Thawing, Counting, and Re-suspension of Enterocytes

Prior to thawing enterocytes, ensure the Biological Safety Cabinet (BSC) is equipped with the following:

- 37°C CERM™
- Ice bucket containing ice
- 4°C HQM™
- Non-coated tissue culture plate, well format as needed
- Serological, P1000 and P200 pipettes and appropriate sterile tips
- Multichannel pipettes and appropriate sterile tips may be used for small well-formats
- Waste container
- Sterile microcentrifuge tubes
- Trypan Blue and DPBS or Medium
- Hemocytometer

Thawing Procedure

1. Warm the 50 mL centrifuge tube of CERM™ in 37°C water bath for 30 minutes. Following the 30-minute incubation at 37°C, transfer CERM™ into the BSC and remove sealing film. Place the tube of HQM™ in the ice bucket containing ice in the BSC.
2. Quickly transfer a vial of cryopreserved enterocytes from the liquid nitrogen storage dewar into the 37°C water bath. Immerse the vial so that the contents are below the waterline and shake gently until the vial is almost completely thawed. The thawing process is approximately 2 minutes. Keep the vial in the water bath while thawing. Removing enterocytes from the water bath prematurely will cause the enterocytes to re-freeze and severely reduce viability. As the last ice crystal is about to disappear, remove the vial from the water bath, spray or wipe the vial with 70% alcohol, and place the vial on ice inside the BSC. It is important to place the vial on ice until you are ready to pour the contents into CERM™ as the cryopreservant is toxic to the enterocytes at temperatures above 12°C.
3. Pour the thawed enterocytes into the CERM™ medium. Use the P1000 with a sterile tip to rinse the vial 3 times with 700 µL of the CERM™ to ensure all the enterocytes have been transferred from the vial into CERM™.
4. Tighten the cap of the CERM™ 50 mL conical, invert the tube gently a few times, and centrifuge at 10 minutes at 100 x g at room temperature for all species of enterocytes.
5. After centrifugation, be careful to keep the pellet intact. Spray or wipe the outside of the conical tube with 70% alcohol and, without inverting the tube, return it to the BSC. Return the cell pellet to the BSC quickly, within 5 minutes after centrifugation, to ensure the cell pellet remains intact.
6. Pour out the supernatant into a waste container in one motion. It is important to pour in one motion and not to reinvert the tube while removing the supernatant. Doing so may disturb the pellet and require re- centrifugation, which may cause cell loss or damage.
7. Add approximately 250 µL of 4°C HQM™ down the side of the tube containing the cell pellet. Gently rock the cell pellet with the media until the cell pellet is dispersed and the cells are re-suspended. Do not vortex or shake vigorously. Keep the cell suspension on ice.

IVAL | 9221 Rumsey Road | Suite 8 | Columbia, Maryland 21045 USA| Tel: +1(410)869-9037 | Fax: (410)869-9034
IVAL | 389 Main Street | Suite 301-304 | Malden, Massachusetts 02148 USA| Tel: +1(781)397-9300 | Fax: (781)397-9309
www.invitroadmet.com

Thawing, Counting, and Re-suspension of Cryopreserved Enterocytes

In Vitro ADMET Laboratories, LLC

Counting Procedure

8. Count the enterocytes using the Trypan Blue exclusion method. Prepare enterocytes for counting (25 µL DPBS or Medium + 25 µL Trypan Blue + 50 µL cell suspension) and mix the contents by inverting the tube. Load the hemocytometer by applying 10 µL from the microcentrifuge tube. Count using 10X magnification and complete the calculations in the following section. 25 µL of DPBS or Medium and 25 µL Trypan Blue and 50 µL of cell suspension create a dilution factor of 2 for the calculation below. Please note, enterocytes tend to clump and counting can be difficult at times. When aggregates are counted, attempt to count for every individual cell that can be seen as part of the aggregate.
9. Approximately 300 µL of residual volume will change the cell suspension volume. Consider re-measuring the cell suspension volume or adding 300 µL to the final volume for an accurate cell count.
10. Adjust enterocytes to a 3.0 cell density and proceed according to specific experimental guidelines.

Calculations

Cell Count Information

Cell Count Dilution	_____	Viable Cells	_____
# of Quadrants	_____	Non-Viable Cells	_____
# of Vials	_____	Total Cells	_____

Viability

_____(Viable Cells) / _____ (Total Cells) x 100 = _____%

Cell Density

_____(Viable Cells) / _____(# of Quadrants) x 10,000 x _____(Dilution) = _____x 10^6 cells/ mL

Viable Yield

_____10^6 cells/mL x _____mL (Cell Suspension Volume) = _____x 10^6 cells

Total Volume from Species Specific Cell Density

_____x 10^6 cell (Viable Yield) / _____ x 10^6 cells/mL (Optimal Cell Density) = _____ (mL) Total Volume

Adjust the Cell Concentration Volume

_____ (mL) Total Volume - _____ (mL) Cell Suspension Volume = _____ (mL) Volume of Media to add

Lot Specific Information

To inquire about our products and services or for technical questions please contact:
In Vitro ADMET Laboratories by phone at +1 (866) 458-1094 or +1 (410) 869-9037 or email at info@invitroadmet.com

IVAL | 9221 Rumsey Road | Suite 8 | Columbia, Maryland 21045 USA| Tel: +1(410)869-9037 | Fax: (410)869-9034
IVAL | 389 Main Street | Suite 301-304 | Malden, Massachusetts 02148 USA| Tel: +1(781)397-9300 | Fax: (781)397-9309
www.invitroadmet.com