Cell Retrieval Kit for Cell-Mate3D™ Standard/Large and µGel40™ Kits Technical Data Sheet #RET-1001

Isolate cells from your Cell-Mate3D™ cultures for downstream applications such as Flow Cytometry or culture maintenance.

CONTENTS: Enzyme Blend: Hyaluronidase (CAS# 37326-33-3) and Chitosanase (CAS# 51570-20-8) blend.

STORAGE/HANDLING: Store at -20°C for up to 1year shelf life.

SAFETY: Use appropriate personal protective equipment when handling enzymes.

STABILITY: Product has a 1year shelf life (unused dry blend enzyme) if stored at -20°C. One vial of the CR Kit can process as many as 5 µGel 40 samples. However, in estimating the number of CR kits required for your application, one must consider how you intend to use the CR kit. Once a vial is reconstituted the enzymes are unstable and must be used within 8 hours and cannot be stored to be used later or freeze/thawed. **Vials are single use only.** If you want to release cells from one µGel on Monday and another µGel on Wednesday, 2 vials would be needed.

ASSESING VIABILITY: Viability should be assessed using Calcein AM and Ethidium Homodimer 1, for example use Molecular Probes Live/Dead Cell Imaging Kit[1] (Thermo Fisher, Cat# R37601). Trypan staining is not suitable for use after cell retrieval because Trypan stains trace amounts of matrix material on the cell membrane.

MATERIALS AND REAGENTS REQUIRED

1) 15 ml conical tube
2) 0.22um syringe filter
3) 24-well cell culture plate
4) 100 µm cell strainer
5) P-1000 pipet and tips
6) Phosphate buffered saline (PBS)
7) Bovine Serum Albumin (BSA)
8) Cell culture media specific to cell type being used
9) 40µL gel embedded with cells (µGel 40™ matrix)
10) Optional: Calcein AM/EtBr Live/Dead Staining Kit

PROTOCOL:

[1] Formerly Invitrogen/Life Technologies brand

1) Prepare the **enzyme dilution buffer**: 1mg/ml BSA in PBS.
2) Reconstitute one vial of **dry enzyme blend** in 1mL of enzyme dilution buffer from step 1.
3) Then prepare a stock solution by adding 200µL of reconstituted enzyme blend from step 2 to 4.8mL of complete cell culture media (total of 5mL). Filter-sterilize the solution with a 0.22µm syringe filter. Allow solution to equilibrate to room temperature. This stock solution will be enough to digest approximately 5 individual µGel 40 matrices.
4) Using a 24-well plate, digest one 40µL matrix with 1000µL of the sterilized enzyme solution prepared in step 3 in a 37°C CO2 incubator for 15 minutes. **PLEASE NOTE:** Be sure that the matrix is completely covered by the sterile enzyme solution as uncovered matrix may not completely dissociate. After 3 minutes of incubation, gently break or begin to pull apart the matrix using a pair of pipette tips. Repeat every 3-4 minutes. After 15 minutes, pipet up and down repeatedly until most of the matrix goes into solution. Some small fragments may remain.
5) Upon Cell-Mate3D matrix digestion, insert the 100 µm cell strainer into a 15ml conical tube and strain the digested product. (The Protocol has been optimized to use a 100 µm strainer)
6) Wash the remaining digested matrix by pipetting 7mL of media or PBS over the mixture while moving the pipette back on forth on the cell strainer. Use 3 mL of media or PBS to wash the well of the 24-well plate and strain this as well.
7) Pellet the cells at 120G for 5 min.
8) Decant the supernatant and culture or process cells as desired.
    a. If preparing cells for Flow Cytometry, filter your samples before analysis to prevent clogs. **See section below on **Staining for Flow Cytometry using Histopaque**
9) Some cultures (greater than 30 days in culture) may require longer than 15 minutes incubation time. See protocol details in section 9 a/b/c
    a. Depending on age of culture, a longer incubation time of 20-30 minutes or may be required
    b. If necessary increasing the amount of reconstituted enzyme blend from 200µl to 300 µl, 400µl, or 500 µL while keeping the final volume at 5mL can improve time of degrading the matrix.
    c. 30-day cultures with additional cells, larger clusters of cells and excess ECM (like collagen) may need secondary digestion requiring the use of collagenase or trypsin buffers to isolate individual cells and dissociate other ECM developed in the culture such as collagen.

TROUBLE SHOOTING GUIDE:

1) If experiencing excess cell death, shorten the incubation period to 10 minutes and/or use gentler pipetting methods.
2) If complex, tissue-like structures (i.e. organoids) have formed in your Cell-Mate3D™ cultures, those structures may not pass through the filter and can be collected off the filter top for further treatment.
3) Long term cultures (i.e. 30 days) may require longer incubation times (like 30-45min) given higher cell density and added ECM from cells in matrix.
    a. A secondary digestion using collagenase or trypsin buffers to dissociate large cell clusters into individual cells and ECM (i.e. Collagen) that may have developed in the culture.
    b. A longer incubation time of 20-30 minutes to ensure that the fragments are broken up into smaller pieces.
    c. Increase the amount of reconstituted enzyme blend concentration to improve time of degrading the matrix.
    d. A combination of two or more of these recommendations as this will vary depending on the cell line(s).
4) Repeat cell straining with additional secondary washes to improve higher yield.
5) Protocol has been optimized to be used with a 100µm cell strainer, we do not recommend using smaller strainer sizes as this may lower your cell retrieval yield.

****Staining for Flow Cytometry including use of Histopaque Kit, we recommend acquiring a Histopaque Kit in order to clean up fragment matrix from cells**

1) Transfer cell pellet sample from step 8 into the FACs tube with cell strainer cap.
2) Wash out the tube that contained the cell pellet with 2.0 mL of FACs buffer and run through strainer cap.
3) Transfer contents (about 2mL) into another FACs tube with 2mL of Histopaque.
4) Spin at 60G for 4 mins. Harvest the interphase and transfer into another FACs tube (refer to Histopaque Kit Protocol for identifying interphase layer).
5) Bring up to 3mL with FACs buffer and spin for 1200rpm 5 min.
6) Decant the supernatant. Add 1µL of human IgG, vortex and incubate for 15 mins.
7) Mix desired antibodies (i.e. for 1 Million target cell count). Scale down as needed.
    a. 2uL CD3 FITC
    b. 1.5uL CD8 PE
    c. 2uL CD4 APC

Prepare Controls – for Flow Cytometry

8) For Isotype controls, use leftover target cells from the wells. Use similar amounts of antibodies for the Isotype controls as above.
9) Directly add 5.5µL of the cocktail to each reaction and incubate for 40 mins on ice. Keep away from light.
10) Wash twice with FACs buffer (270G 5 mins).
11) Decant supernatant and fix with 400µL of 2% Paraformaldehyde.

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