CULTREX® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

3D Culture Cell Harvesting Kit

For harvesting and lysate preparation from 3D Culture for Western analysis

Sufficient reagents for twenty 35 mm dishes

Catalog #: 3448-020-K

Cultrex® 3D Culture Cell Harvesting Kit

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20 Samples

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I. Quick Reference Procedure for the CULTREX® 3D Culture Cell Harvesting Kit: Read all *Instructions for Use* prior to using this kit.

Instructions for isolating cells from 3D Culture Matrix™ BME or 3D Culture Matrix™ Laminin I:

1. For each 35 mm plate, dilute buffers, and chill overnight at 4°C.
   a. Dilute 5 ml of 10X Wash Buffer to 50 ml with dH_2O.
   b. Dilute 5 ml of 10X Cell Harvesting Buffer to 50 ml with dH_2O.
   c. Keep buffers on ice throughout procedure.
2. Working on ice, aspirate cell culture media, and gently wash dish three times with 10 mL of 1X Wash Buffer.
3. After final wash, aspirate buffer, and add 6 mL of 1X Cell Harvesting Buffer to dish.
4. Resuspend cells and matrix in 1X Cell Harvesting Buffer by gently pipetting up and down using a serological pipet.
5. Once resuspended, transfer contents to a 15 mL conical tube.
6. Wash the dish with an additional 6 mL of 1X Cell Harvesting Buffer, and transfer to the same 15 mL conical tube.
7. Seal the tube, and place on its side within an ice container.
8. Gently shake container for 30 minutes on an orbital lab shaker.
9. Centrifuge conical tube at 200 *x g* for 5 minutes, and aspirate supernatant. Samples may be washed with additional 1X Cell Harvesting Buffer if needed.
10. Reagents are supplied for preparation and normalization of lysates for western blot; alternatively, cells may be processed for RNA or DNA extraction using Trizol™.

II. Background

3D Cultures exhibit cellular behaviors and morphologies similar to those seen *in vivo*, however, the adaptation of these models for studying biochemical processes has been impeded by the challenge of separating intact cells from extra-cellular proteins comprising the hydrogel. Commonly, proteases are employed to degrade these extracellular proteins, however, proteases also degrade proteins on the cell surface and protease activity may carry over into lysate preparations. Non-enzymatic methodologies have also been described for depolymerizing extracellular matrix proteins, although the implementation of these protocols remains problematic for some researchers. Trevigen's **Cultrex® 3D Culture Cell Harvesting Kit** provides an optimized and standardized solution for the isolation and normalization of cell lysates from 3D Culture™ BME or Laminin I for subsequent biochemical analysis.

III. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of these products may not yet have been fully investigated; therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.
3. The **Cultrex® 3D Culture Cell Harvesting Kit** contains reagents that may be harmful if swallowed, or come in contact with skin or eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Material safety data sheets are available on request.

IV. Materials Supplied

Component	Quantity	Storage	Catalog#
10X Cell Harvesting Buffer	100 ml	Room Temp	3448-020-01
10X Cell Wash Buffer	100 ml	Room Temp	3448-020-02
Sample Buffer	10 ml	Room Temp	3448-020-03
10X Loading Buffer	1 ml	Room Temp	3448-020-04
Anti-h/m G3PDH	20 ul	-20°C*	2275-PC-020

*Store in a manual defrost freezer.

V. Materials/Equipment Required But Not Supplied

Equipment

1. 1 - 20 µl pipettor, 20 - 200 µl pipettor, and 200 - 1000 µl pipettor
2. Low speed swinging bucket 4°C centrifuge and tubes for cell harvesting
3. 4˚C storage
4. ice bucket/container
5. pipette helper
6. timer
7. Orbital shaker

Reagents

1. Deionized water
2. Antibody blocking buffer (TBST or 5% milk)

Disposables

1. 15 ml tubes
2. 1 - 200 µl and 200 - 1000 µl serological pipette tips
3. 1, 5 and 10 ml serological pipets
4. gloves

VI. Reagent Preparation

1. **10X Cell Harvesting Buffer**
   Dilute 1:10 in deionized water, and chill to 4°C prior to use.
2. **10X Cell Wash Buffer**
   Dilute 1:10 in deionized water, and chill to 4°C prior to use.
3. **10X Loading Buffer**
   Dilute 1:10 in cell lysates for western blotting.
4. **Rabbit Anti-G3PDH Polyclonal Antibody**
   Dilute 1:1000 in blocking buffer, such as TBST, 5% milk, and use immediately. Store unused antibody in aliquots at -20°C. Avoid freeze/thaws.

VII. Assay Protocol

A. Cell Harvesting

Optimal depolymerization of BME or Laminin I requires at least a five fold excess of 1X Cell Harvesting Buffer and is more efficient with larger ratios. The reaction should be performed in 15 ml conical tubes for optimal cell pelleting. Larger volumes and high density hydrogels may require dividing samples into multiple tubes for optimal depolymerization.

1. For each 35 mm plate, dilute buffers, and chill overnight at 4°C.
   a. Dilute 5 ml of 10X Wash Buffer to 50 ml with dH_2O.
   b. Dilute 5 ml of 10X Cell Harvesting Buffer to 50 ml with dH_2O.
   c. Keep buffers on ice throughout procedure.
2. Working on ice, aspirate cell culture media, and gently wash dish three times with 10 mL of 1X Wash Buffer. Be very careful not to disturb hydrogel; if the hydrogel appears to break up, transfer washes to a 15 ml conical tube, centrifuge off wash solution, and use this tube in step 15.
3. After final wash, aspirate buffer, and add 6 ml of 1X Cell Harvesting Buffer to dish.

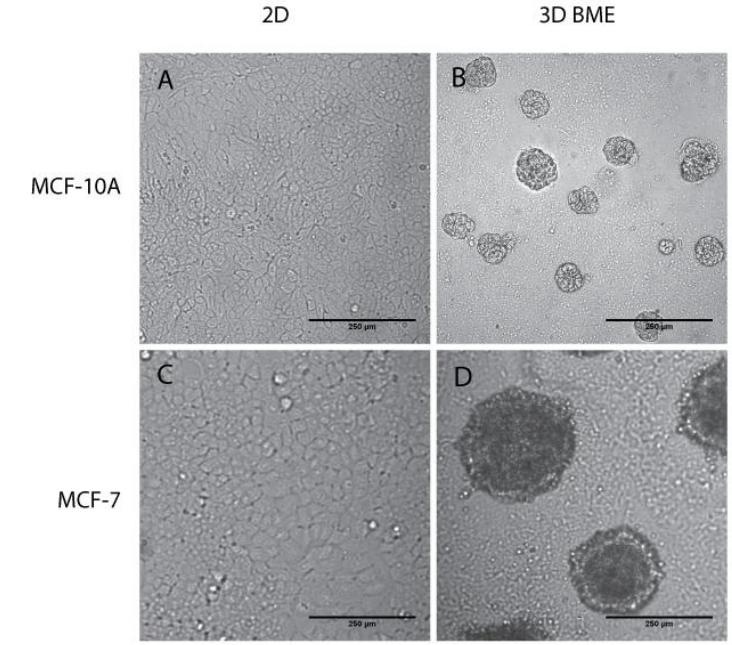

Figure 1. Morphology of MCF-10A (A,B) and MCF-7 cells (C, D) in traditional 2D culture and 3D BME culture, scale = 250 µm.

4. Resuspend cells and matrix in Cell Harvesting Buffer by gently pipetting up and down using a serological pipet.
5. Once resuspended, transfer contents to a 15 mL conical tube.
6. Wash the dish with an additional 6 mL of 1X Cell Harvesting Buffer, and transfer to the same 15 mL conical tube.
7. Seal the tube, and place on its side within an ice container.
8. Gently shake container for 30 minutes on an orbital lab shaker.
9. Centrifuge conical tube at 200 *x g* for 5 minutes at 4°C, and aspirate supernatant. If a thick layer of hydrogel is still visible, then wash with additional 10 ml 1X Cell Harvesting Buffer, centrifuge at 200 *x g* for 5 minutes at 4°C, and aspirate supernantant; repeat if necessary. It is important to keep cell samples and Cell Harvesting Buffer on ice during processing.
10. Reagents are supplied for preparation and normalization of lysates for western blot; alternatively, cells may be processed for RNA or DNA isolation using Trizol™.

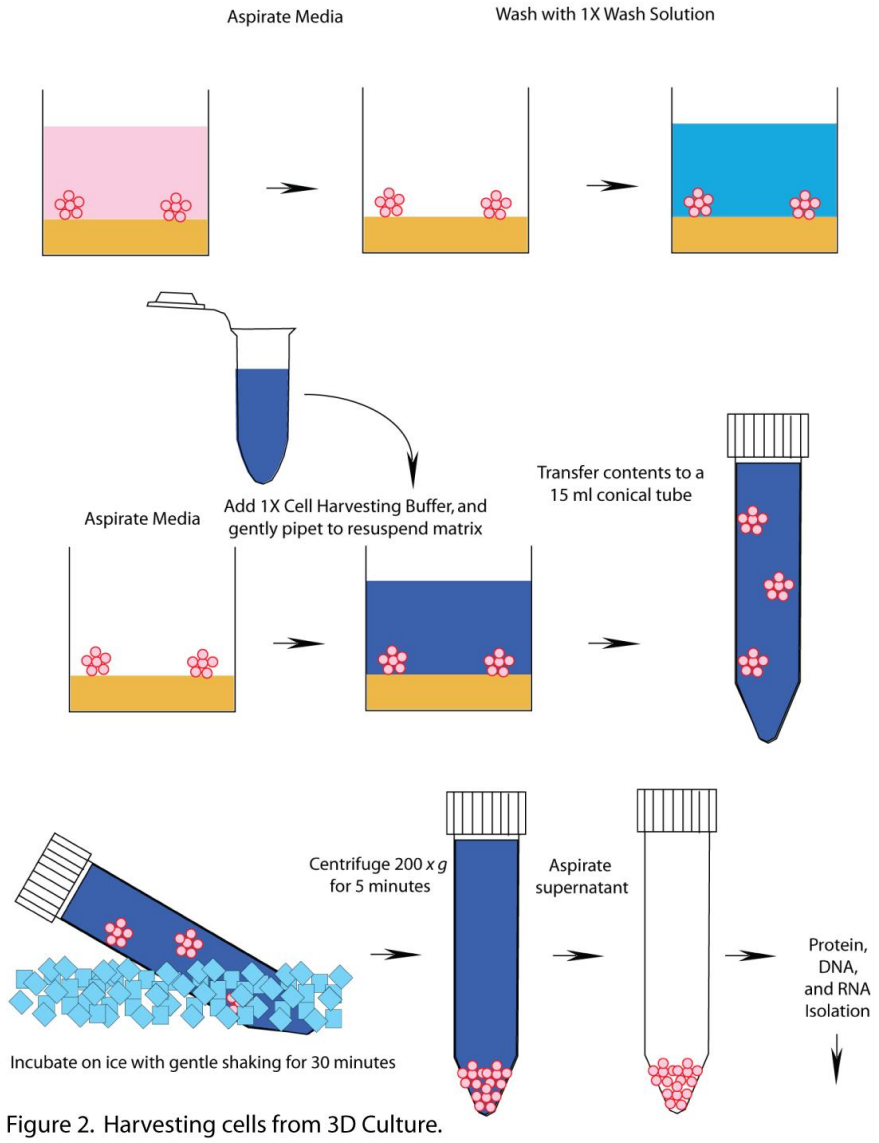

Figure 2. Harvesting cells from 3D Culture.

B. Lysate preparation

1. Resuspend cell pellet in 50 µl of Sample Buffer, and pipet up and down to aid in cell lysis. Additional Sample Buffer may be necessary to solubilize large cell pellets. Protease inhibitors may also be added at this time, if needed.

2. Incubate lysates at 95°C for 5 minutes.

3. Assay lysates for protein concentration. OD_{280} and BCA assays have resulted in reproducible results.

4. Determine dilution for lysate mass:

_____ X _____ = _____
Lysate concentration ug/ul Lysate volume (ul) Lysate mass (ug)

5. Determine final volume:

_____ / _____ = _____
Lysate mass (ug) Desired concentration ug/ul Final Volume (ul)

6. Determine volume of 10X Loading Buffer needed:

_____ X 0.1 = _____
Final Volume (ul) 10X Loading Buffer (ul)

7. Determine volume of Sample Buffer Needed:

_____ - _____ - _____ = _____
Final Vol (ul) Lysate Vol (ul) 10X Loading Buffer (ul) Sample Buffer (ul)

   Combine cell lysates with 10X Loading Buffer (step 6) and Sample Buffer (step 7); DTT, β -mecaptoethanol, or other reducing reagents may also be added at this time, if needed.

8. Normalize lysate loading by evaluating sample via Western Blot using a 1:1000 dilution of rabbit anti-G3PDH polyclonal antibody. Antibody must be used immediately after dilution; unused antibody should be stored in aliquots at -20°C.

9. Use normalized volumes for assessing targets of choice.

C. Sample Data:

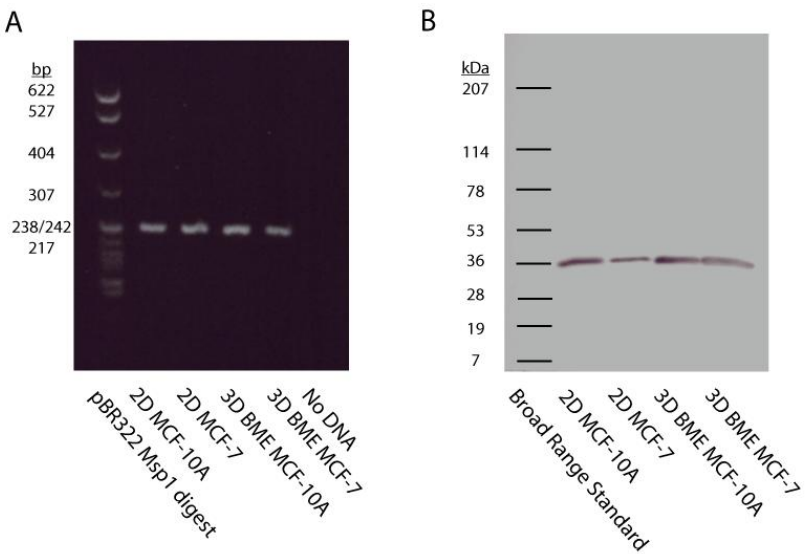

Figure 3. Evaluation of expressiof RNA expression via RT-PCR (A) and protein expression via Western bloting (B) for GAPDH from MCF-10A and MCF-7 mammary epithelial cells isolated from either traditional 2D culture or 3D BME culture.

VIII. Troubleshooting

Problem	Cause	Solution
Poor depolymerization of BME or Laminin	Incorrect temperature.	Cell Wash and Harvesting Buffers must be chilled to 4°C the night before and kept on ice throughout the procedure.
		Cells must be kept on ice and centrifuged at 4°C while harvesting.

Problem	Cause	Solution
Poor depolymerization of BME or Laminin (cont.)	Insufficient ratio of Cell Harvesting Buffer to Matrix.	Divide sample into multiple 15 ml conical tubes, and proceed with cell harvesting.
Poor yield of protein, DNA, or RNA	Poor cell yield	Inadequate cell proliferation in 3D culture as a result of insufficient cell seeding, cell treatment, or cell health.

IX. Appendices

Appendix A. Reagent and Buffer Composition

1. **10X Cell Harvesting Buffer**
   Proprietary formulation containing sodium citrate, EDTA, and glycerol.
2. **10X Cell Wash Buffer**
   213 mM Potassium Phosphate, pH 7.4, 1.45 M NaCl
3. **Sample Buffer**
   12.5 mM Tris-Cl, pH 6.8, 4% SDS, 10% Glycerol.
4. **10X Loading Buffer**
   125 mM Tris, pH 6.8, 4% SDS, 10% Glycerol, 0.1% Bromophenol Blue
5. **Rabbit Anti-G3PDH Polyclonal Antibody**
   A polyclonal antibody prepared from synthetic peptide corresponding to a portion of the human G3PDH sequence. Shown to cross react with mouse G3PDH (other species not tested). Detects ~38 kDa band during Western blot.

Appendix B. Related products available from Trevigen.

Catalog #	Description	Size
3445-096-K	**3D Culture 96 Well BME Cell Proliferation Assay**	96 samples
4890-025-K	TACS™ MTT Cell Proliferation Assay	2500 tests
4891-025-K	TACS™ XTT Cell Proliferation Assay	2500 tests
4895-50-K	TACS™ 2 Hoechst CPA1 Kit (Vital)	2500 tests
4896-50-K	TACS™ 2 Hoechst CPA2 Kit (Fixed)	2500 tests
4892-010-K	Cultrex® Calcein-AM Cell Viability Kit	1000 tests
4817-60-K	FlowTACS™ Apoptosis Detection Kit	60 samples
4822-96-K	HT TiterTACS™ Assay Kit	96 tests
4830-01-K	TACS™ Annexin V FITC Kit	100 samples
4835-01-K	Annexin V Biotin Kit	100 samples
6300-100-K	DePsipher™ Mitochondrial Potential Assay Kit	100 tests
6305-100-K	MitoShift™ Mitochondrial Potential Assay Kit	100 tests

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