Harvesting Organoids for Biochemical Analysis

3-D cell culture models that resemble the cellular composition and architecture of their tissues of origin represent the next generation of medical research models as they provide physiologically relevant systems for studying human diseases, screening for drug toxicity, and investigating personalized medicines.

These systems, known as organoids or mini-organs, are generated from pluripotent or adult stem cells, which allows them to be passaged, mainly in a 3-D matrix, almost indefinitely. In order to passage or harvest embedded organoids, proteases are employed to degrade the extracellular proteins. However, proteases also degrade proteins on the cell surface, and protease activity may carry over into subsequent cultures or lysate preparations.

Cultrex™ Organoid Harvesting Solution (R&D Systems, Catalog # 3700-100-01) provides a non-enzymatic method for depolymerizing extracellular matrix proteins to allow intact organoids to be harvested for passaging, cryopreservation, or biochemical analysis. Cultrex Organoid Harvesting Solution is compatible with the main biochemical analysis techniques, such as quantitative PCR (qPCR) and Western blotting. This protocol outlines the use of Cultrex Organoid Harvesting Solution for harvesting organoids to analyze changes in gene expression by qPCR or to detect a specific protein of interest by Western blot.

Protocol

Method for Harvesting Organoids Using Cultrex Organoid Harvesting Solution

A visual schematic of the Organoid Harvesting process is shown in Figure 1.

Note: *To extract RNA or prepare total protein lysates, it is recommended to harvest at least 6 domes of BME containing 100 organoids or more.*

1. Discard the culture or differentiation medium and wash the well with 5 mL of cold (2-8 °C) PBS. Incubate the organoids with 5 mL of cold (4 °C) Cultrex Organoid Harvesting Solution for 30 to 60 minutes.

Note: *During this time, the plates should be placed inside a container with ice or in a cold room with gentle shaking in order to achieve maximum depolymerization of the Cultrex BME.*

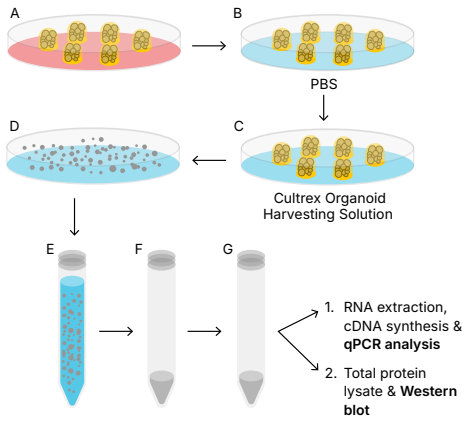

Figure 1. Summary for Harvesting Organoids for Biochemical Analysis. A) Discard medium. B) Wash the organoids with cold PBS. C) Add Cultrex Organoid Harvesting Solution. D) Incubate at 2–8 °C. E) Transfer the organoids to a conical tube. F) Centrifuge the organoids. G) Resuspend the organoids in the appropriate lysis solution for either RNA extraction or protein analysis.

2. Once the matrix is dissolved and the organoids are released, transfer the solution to a conical tube and centrifuge the tube at 500 x g for 5 minutes at 2-8 °C. Discard the supernatant.

Note: *The organoid pellet should be visible in the bottom of the tube, but it may depend on the number of organoids harvested.*

RNA Extraction and cDNA Synthesis

1. Resuspend the organoid pellet in 1 mL of TRIzol®. Total RNA from organoids can be extracted using commercial or standard extraction methods. Use approximately 500 ng of RNA to synthesize cDNA from each sample.

   Note: *For alternative applications, the organoid pellet can be resuspended in a different lysis buffer.*

Quantitative PCR (qPCR)

1. Dilute each cDNA sample 10 times with nuclease-free water for use as a template for quantitative PCR. Set-up 25 µL reactions for qPCR based on the table below.

TABLE // 01

Preparation of qPCR Reactions

Reagent Name	Volume (25 µL)
2x iQ™ SYBR® Green Supermix (Bio-Rad)	12.5 µL
Forward and Reverse qPCR Primer Mix (500 nM)	0.5 µL
Nuclease-free water	9.5 µL
Diluted cDNA	2.5 µL
Total	25 µL

2. Measure each sample in triplicate using a thermocycler.

   Note: *All primer pairs were ordered from IDT DNA technologies. Beta-2 microglobulin was used as internal control.*

Total Protein Lysate Preparation

1. Resuspend the organoid pellet in 1 mL of cold (2–8 °C) RIPA buffer (50 mM Tris-HCL, pH 7.4, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, 150 mM NaCl, 5 mM EDTA, 50 mM NaF) plus 1X Halt™ Protease Inhibitor Cocktail, 1mM PMSF, and 1mM Benzamidine-HCL.
2. Working on ice, vortex the samples and lyse the cells either by sonication of the lysates, or by passing them through a 25 gauge needle attached to a 1 mL syringe.
3. Quantify the protein concentration and prepare the samples as follows: protein concentration 0.3 µg/µL, 1X NuPage® LDS buffer and 100 mM DTT. Boil the samples for 2 minutes and load 4.5 µg of total protein in each lane.

Results

qPCR

The qPCR results are shown in Figure 2. Lgr5, a G-protein coupled receptor, expressed mainly in stem cells is reduced after only 2 days of differentiation. This result is expected as the stem cell population decreases, and new crypts and villi start to form. Conversely, the expression of Defa5, Muc2, and Tff3, all markers of intestinal differentiated cell types were increased over time.[1, 2]

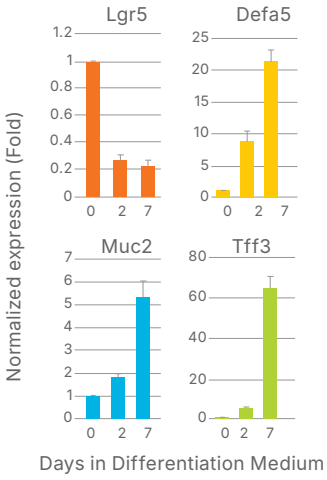

Figure 2. Quantitative PCR Analysis. At days 0, 2, and 7 of differentiation, mouse small intestine organoids were harvested and gene expression of the enteric markers Lgr5, Defa5, Muc2 and Tff3 was evaluated by qPCR.

Western Blot

Control and differentiated mouse small intestine organoids were harvested as indicated above and resuspended in RIPA buffer plus protease inhibitors. A Western blot for beta III Tubulin expression is shown in Figure 3 and demonstrates the compatibility of Cultrex Organoid Solution with this technique.

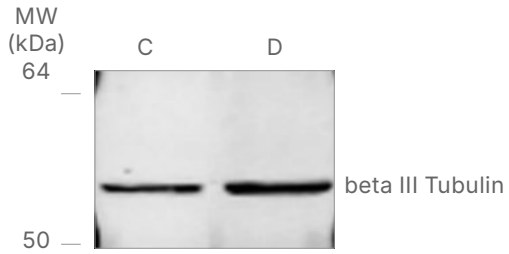

Figure 3. Western Blot Analysis of Mouse Small Intestine Organoids. Mouse small intestine organoid pellets were harvested with Cultrex Organoid Harvesting Solution and resuspended in RIPA buffer. A Western blot against beta III Tubulin for control (C) and differentiated (D) organoids was performed.

References

1. VanDussen, K. L. *et al.* (2015) Gut **64**:911.
2. Yin, X. *et al.* (2014) Nat. Methods **11**:106.

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