Cultrex™ Organoid Harvesting Solution

Catalog Number: 3700-100-01 (100 mL)

PRODUCT DESCRIPTION

Organoid cultures exhibit cellular behaviors and morphologies similar to those seen *in vivo* (1-6), however the adaptation of these models for studying biochemical processes has been impeded by the challenge of separating intact organoids from extracellular proteins comprising the hydrogel. Proteases are often employed to degrade these 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 provides a non-enzymatic method for depolymerizing extracellular matrix proteins to allow for harvesting of intact organoids for passaging, cryopreservation, or biochemical analysis.

INTENDED USE

Cultrex Organoid Harvesting Solution is ready-to-use solution designed for the harvesting of intact organoids embedded in culture matrices, such as Cultrex® Basement Membrane Extracts (BME).

STORAGE CONDITIONS

Product is stable for at least 2 months from the date of receipt when stored at 2-8 °C. Keep sterile. See lot specific Certificate of Analysis for expiration.

PRECAUTIONS

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

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ORGANOID HARVESTING PROCEDURE

1. Working on ice, aspirate cell culture media and gently wash each well with 10 volumes of cold (2-8 °C) PBS (Table 1). Be careful not to disrupt basement membrane matrix containing organoids.
2. Aspirate the PBS, and add 10 volumes of cold (2-8 °C) Cultrex™ Organoid Harvesting Solution to each well (Table 1).

PLATE TYPE	VOLUME OF BASEMENT MEMBRANE MATRIX	VOLUME OF PBS AND ORGANOID HARVESTING SOLUTION
96-well plate	5 µL	50 µL
48-well plate	25 µL	250 µL
24-well plate	50 µL	500 µL

Table 1: Suggested working volumes of PBS and Cultrex Organoid Harvesting Solution.

3. Incubate the plate at 2-8 °C or on ice for 30–90 minutes with moderate shaking. This incubation is complete when the basement membrane matrix dome is no longer visible at the bottom of the well and the organoids are seen floating at the bottom of the well.
   Note: *Dislodging the dome with a cell scraper or pipet may accelerate this process.*
4. Once the matrix depolymerizes, transfer contents of the well into a tube on ice. Single wells may be transferred to a microtube while multiple domes may necessitate a 15 mL or 50 mL conical tube.
5. Centrifuge the tube at 500 x g for 5 minutes at 2-8 °C in a swinging bucket rotor to pellet the organoids. Aspirate the supernatant.
6. Wash organoids with 10 volumes of cold (2-8 °C) PBS, and repeat centrifugation at 500 x g for 5 minutes at 2-8 °C in a swinging bucket rotor to pellet the organoids. Aspirate the PBS.
7. Isolated organoids may be:
   a. Resuspended in basement membrane matrix for further organoid culture.
   b. Resuspended in freezing medium for cryopreservation.
   c. Processed for biochemical analysis (such as RT-PCR, MS-PCR, sequencing, Western Blot, ELISA, or IHC).

REFERENCES

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3. Salahudeen, A.A. and C.J. Kuo (2015) Nat. Med. **21**:215.
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