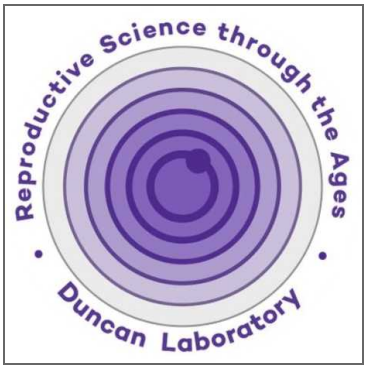

NOV 09, 2023

DOI:
dx.doi.org/10.17504/protocols.io.x54v9pwwmg3e/v1

Protocol Citation: hannah.anvari, francesca.e.duncan duncan 2023. Generating supplement-free conditioned media for proteomic analysis following human ovarian tissue culture.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9pwwmg3e/v1

Protocol status: Working
We use this protocol and it's working

Generating supplement-free conditioned media for proteomic analysis following human ovarian tissue culture.

hannah.anvari[1], francesca.e.duncan duncan[1]

[1]Northwestern Medicine OBGYN, Northwestern University

pooja.devrukhkar

ABSTRACT

Purpose: This protocol is intended for use in generating supplement-free conditioned media for proteomic analysis. This protocol supplements the steps detailed in the processing and culture of human ovarian tissue for induced senescence as detailed in protocol dx.doi.org/10.17504/protocols.io.3byl4qbdjvo5/v2

GUIDELINES

Guidelines for researchers working with human specimens

Researchers will adhere to all safety and training protocols required by the Northwestern Medicine/Northwestern University including but not limited to:

1. Biosafety Certification
2. Bloodborne Pathogens Certification
3. Working with Formaldehyde Certification
4. Collaborative Institutional Training Initiative (CITI program) certification

Created: Nov 09, 2023

Last Modified: Nov 09, 2023

PROTOCOL integer ID: 90746

Keywords: Cellular Senescence, Human Ovary, Doxorubicin, Conditioned Media, Proteomics, Reproductive Aging

Funders Acknowledgement:
SenNet
Grant ID: U54AG075932
SenNet
Grant ID: UG3CA268105

MATERIALS

1. ORIGIO Handling IVF Medium (ORIGIO, #83100060)
2. Disposable Scalpel, Sterile, No. 10 and No. 22 (Fisher Scientific, 12-460-456)
3. 15 mL conical tubes (Fisher Scientific, 2610L35) for tissue collection.
4. Gibco™ DPBS, calcium, magnesium (Fisher Scientific, 14-190-250)
5. 60 mm Dish, Non-Treated, Corning™ Falcon™ Bacteriological Petri Dishes with Lid (Fisher Scientific 08-772-12 or equivalents) for processing
6. Stadie-riggs tissue slicer (discontinued)
7. Stadie-Riggs tissue slicer/microtome blades (Thomas Scientific 6727C18)
8. Stadie-Riggs tissue slicer blade handle (Thomas Scientific 6727C25)
9. Plastic Discs (Fisher Scientific, 1018001)
10. MEM Alpha (1X) + GlutaMAX (Thermo, 32561037)
11. Human serum albumin (Cooper Surgical Inc, ART-3003)
12. Fetuin (Sigma, F3385-25G)
13. Insulin-Transferrin-Sodium Selenite Supplement (Sigma-Aldrich, I1884-1VL)
14. Doxorubicin (Fisher Scientific, 22-521-0)
15. Millicell Cell Culture Insert, 12 mm, hydrophilic PTFE, 0.4 µm (Millipore Sigma, PICM01250)
16. Falcon® 24-Well Flat-Bottom Plate, Tissue Culture-Treated (Fisher Scientific, 353047)
17. Invitrogen™ RNase-free Microfuge Tubes (ThermoFisher Scientific, AM12400)

SAFETY WARNINGS

Researchers will wear personal protective equipment (PPE) when working with human specimens which include gloves, mask, eye protection, and lab coat.

ETHICS STATEMENT

Human ovarian tissue procurement and processing for ovarian explant cultures adhere to the approved IRB protocol under NU (NU12G09) for the collection of human ovarian tissue through Northwestern Medicine.

Wash steps to generate supplement-free conditioned media

1 Human ovarian tissue is processed and cultured according to an established protocol (dx.doi.org/10.17504/protocols.io.3byl4qbdjvo5/v2) upto Day 10.

2 On day 10 of culture, two complete media changes are performed for each well as follows (Fig. 1):

a. In the original plate, each well is washed twice with pre-equilibrated basal media (MEM Alpha (1X) + GlutaMAX (Thermo, 32561037) without additional supplements).
b. From the original plate, one transwell is removed at a time and released back into a wash dish containing pre-equilibrated basal media.
c. The tissues are reloaded onto new transwells and moved into a new plate containing pre-equilibrated basal media (The plate map matches the original plate). One well is reloaded at a time.
d. The new plate is reloaded into the incubator for an additional 24 hours.

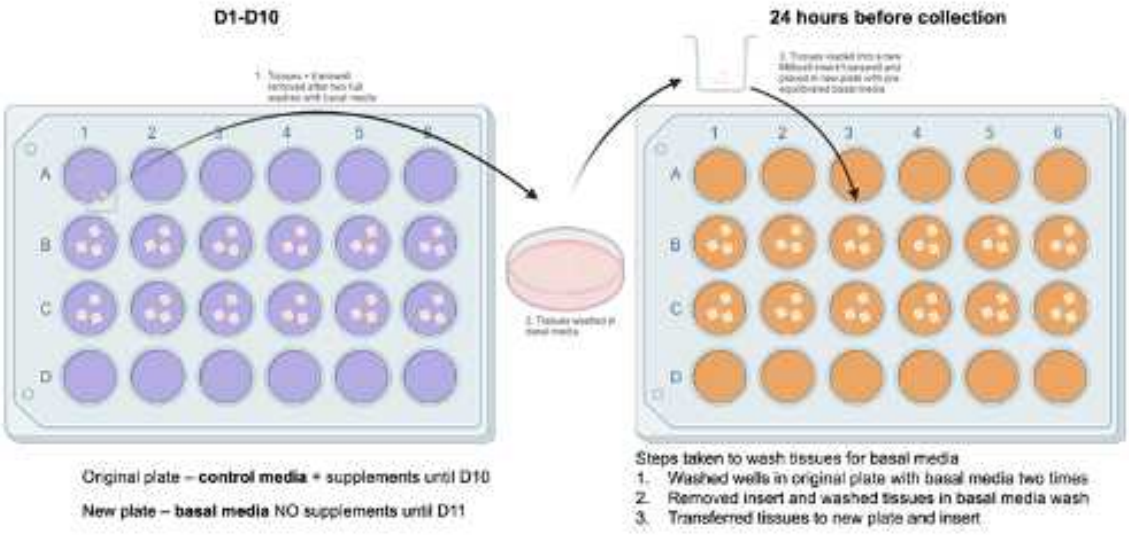

Fig. 1: Schematic showing steps to generate supplement-free conditioned media for proteomic analysis.

3 When the culture period ends, all the conditioned media is collected into microcentrifuge tubes and snap-frozen over dry ice. The tubes are stored at -80°C until further use.

4 The tissues from the inserts are collected by carefully peeling away the mesh bottom with sterile forceps. The edge of the transwell is held with curved forceps and the edge of the membrane is pierced with straight forceps such that it breaks away from the plastic edge. The remaining membrane is then peeled away from the edge.

5 The explants are then fixed according to an established protocol (dx.doi.org/10.17504/protocols.io.x54v9pyz1g3e/v1).

6 If freezing the tissues, the mesh bottom is placed into a dish of PBS and the tissues are carefully moved into a dry 1.5 mL microcentrifuge tube using a similar technique used to load the tissues onto the wells: take up some PBS and tissue (100-200 µL) into the cut tip of a 1000 µL pipette tip. Expel the contents into a dry 1.5 mL microcentrifuge tube and then remove excess PBS with a 200 µL pipette tip before placing them on dry ice. Store at -80°C.