THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Conditioned Medium Protocols: Factors Secreted from Cultured Lines

Materials:

Reagent name	Supplier	Cat No.	Stock solution	Final Conc
Advanced DMEM/F12	Invitrogen	12634-028		
DMEM	Invitrogen	11995-073		
FBS				
GlutaMAX-I	Invitrogen	35050-061	100X	1X
G418	Invitrogen	10131035	50mg/ml	0.4mg/ml
Zeocin	Invitrogen	45-0430	100mg/ml	300ug/ml
Puromycin	Invitrogen	A11138-03	10mg/ml	10ug/ml
Trypsin-EDTA	Invitrogen	25200-056		

Equipment

Flask T75 (Corning #430641U), Centrifuge

Medium

Growth Medium

L-Wnt 3a: DMEM+10% FBS + G-418 (stock is 50mg/ml, add 400ul into 50ml medium, final 0.4mg/ml)

R-Spondin: DMEM+10% FBS + Zeocin (stock is 100mg/ml, add 150ul into 50ml medium, final 300ug/ml)

Noggin: DMEM+10% FBS + puromycin (stock is 10mg/ml, add 50ul into 50ml medium, final 10ug/ml)

Medium for making conditioned medium

Conditioned Medium Made from GF Secreted Cultured Cell Lines Protocol v4-12/5/16 Saved at Data (//storage/bcm-mvm-public)-Lab Estes-Protocols-Enteroids

Conditioned Medium Protocols: Factors Secreted from Cultured Lines

Advanced DMEM/F12+1xGlutaMAX+8%FBS

ATCC L-Wnt-3A Conditioned Medium:

1. Thaw a vial of ATCC L-Wnt3A cell (ATCC CRL-2647) from liquid nitrogen storage.

2. Culture one vial of frozen cell into 1xT75 flasks containing 15ml of L-Wnt3a growth medium. After couple of days, when cells are confluent, split 1:10, add 15ml Growth Medium in 1xT75 and 10ml medium for making conditioned medium in 9xT75

3. For 9 x T75 flasks, harvest medium after 4 days culture. Take off the medium, centrifuge at 1000g (2,990rpm) and sterile filter (0.22uM filter). This is the first batch of medium.

4. Add 10ml fresh medium for making conditioned medium to the 9 flasks and culture for another 3 days.

5. Take off the medium, centrifuge at 1000g (2,990rpm) and sterile filter. This is the second batch of medium. Discard the cells, because they will be overgrown.

6. Mix the first batch and second batch of medium 1:1. This is the Wnt3A conditioned medium.

7. Aliquot 25ml per 50ml conical tube and store at -80c.

R-Spondin Conditioned Medium:

1. Thaw a vial of 293T-HA-Rspol-Fc cells from liquid nitrogen storage. These cells should be obtained from Trevigen that has licensed the right to manufacture, market, and distribute the R-Spondin1 cell line to the research community. They will provide a product release document that shows data from the production of R-Spondin-1 for organoid culture. Below is ordering information.

Ordering Information ***No MTA required, it is covered in the Instructions for Use***
Catalog# 3710-001-01

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Product Name: Cultrex HA-R-Spondin1-Fc 293T Cells
Size: 1 vial, 1x 10^6 cells (0.5 ml)
Price: $490.00

2. Divide one vial of frozen cells into 1 x T75 flasks containing 15ml of growth medium for R-Spondin. After 3-4 days, when cells are 80% confluent, split 1:5. Add 15ml media containing zeocin to 1 x T75 flask and 15 ml media without zeocin to 4 x T75 flasks. Culture for another 2-3 days until 80% confluent.

3. Trypsinize 4 x T75 flasks without Zeocin with 1:3 diluted Trypsin-EDTA in PBS (from 0.25% Trypsin-EDTA, at room temperature for approximately 30 second or until cell start detach from the flask) neutralize with DMEM+10% FBS and centrifuge at 200g (1,338rpm). Remove the trypsin and medium, divide the cell pellet into 10 x T75 flasks each containing 20ml medium for making conditioned medium.

4. Harvest after one week. Pool medium from all flasks into centrifuge tube, centrifuge at 1000g (2,990rpm) for 10 minutes and filter supernatant through a .22uM filter.

5. Aliquot 10ml per 15ml tube and store at -80c.

6. 1xT75 with media containing Zeocin from step 2, passage as described in steps 3-5.

Note: when you trypsinize 293T-HA-Rspol-Fc cells, use original (0.25%) trypsin 1:3 diluted with PBS (-Mg, -Ca).

Noggin Conditioned Medium:

1. Thaw a vial of 293-Noggin cells from liquid nitrogen storage [obtained from Dr. Vanessa Muncan and Dr. Gijs van den Brink - no MTA required but need to quote his paper describing these cells when we publish our work; (Heijmans J, van Lidth de Jeude JF, Koo BK, Rosekrans SL, Wielenga MC, van de Wetering M, Ferrante M, Lee AS, Onderwater JJ, Paton JC, Paton AW, Mommaas AM, Kodach LL, Hardwick JC, Hommes DW, Clevers H, Muncan V, van den Brink GR. 2013. ER stress

causes rapid loss of intestinal epithelial stemness through activation of the unfolded protein response. Cell Rep. 2013 Apr 25;3(4):1128-39)].

2. Divide one vial of frozen cells into 4 x T75 flasks containing 15ml of growth medium for Noggin. After 2-3 days or when cells reach 80% confluent, split 1:5. Add 15ml media containing puromycin to 1 x T75 flask and 15 ml media without puromycin to 4xT75. Culture for another 2-3 days until 80% confluent.

3. Trypsinize 4 x T75 flasks without puromycin with 1:3 diluted Trypsin-EDTA in PBS (from 0.25% Trypsin-EDTA, at room temperature for approximately 30 second or until cell start detach from the flask) neutralize with DMEM+10% FBS and centrifuge at 200g (1,338rpm). Remove the trypsin and medium, divide the cell pellet into 10 x T75 flasks each containing 20ml medium for making conditioned medium.

4. Harvest after one week. Pour all medium into centrifuge tube, centrifuge at 1000g (2,990rpm) for 10 minutes, and filter the medium through .22uM filter.

5. Aliquot at 10ml per 15ml tube and store at -80c.

6. 1xT75 with medium containing puromycin from step 2, passage as described in steps 3-5.

Note: when trypsinizing 293 Noggin cells, use original (0.25%) trypsin 1:3 diluted with PBS (-Mg, -Ca), otherwise the original 0.25% trypsin is too strong for the cells and the cells won't grow well.