MOC2 细胞系

Uppaluri Lab Tissue Culture Protocols for MOC cell lines Updated 12.12.16

Materials needed (see attached IMDM protocol for reagents needed to make IMDM MOC line media)

Sigma Aldrich ： DMSO:D2650-100ml

FisherScientific:

T150Flasks ： 07-200-64

T75 Flasks ： 10-126-37

Cryovials ： 03-374-059

45um filters: ： 09-754-21

05% Trypsin ： sh30236.01

25% Trypsin ： sh30042.01

Indolent Lines – MOC1, 22

Aggressive Lines – MOC2

Thawing cell lines

1. Add 21ml IMDM MOC line media to a T150 before thawing (or 10ml to a T75 if wanting to thaw into aT75)

2. Remove cryovial from liquid nitrogen, spray vial with 70% alcohol to clean it.

3. Hold bottom-half of cryovial in 37Cwaterbath (without letting lid touch water, to avoid contamination) until there is a small chunk of ice left floating.

4. Spray cryovial again with ETOH and place in hood. Pipette 1ml of media to the

1ml of cells and add these 2ml to the T150 that already contains media (to make 22ml total for one T150).

5. Take some media already in T150 flask and rinse the cryovialand plate this to ensure you have all the residual cells left in the cryovial.

Freezing cell lines:

Work quickly, as DMSO is toxic to cells

For each T150 flask with 70-80% cell confluence, freeze 3-4 vials.

1. Harvest cells from T150 as seen below

2. Spin down into pellet in 15ml conical tube (1000 RPM x5 min)

3. Dump out supernatant

4. Tap 15ml conical tube toresuspend cells

5. Add 1.5ml of IMDM MOC line media, reconstitute cells in media – keep on ice

6. Add 1.5 ml of freezing media dropwise slowly while tube is on ice

To make freezing media – 20% DMSO in IMDM MOC line media. Ex: For 20ml stock - add 16ml IMDM MOC line media and 4ml DMSO. Syringe filter using .45um filter

7. Aliquot 1ml each to 3 cryovials

8. Store in -80C for no more than 2 weeks.

9. Place into liquid nitrogen within 1-2 weeks.

Note: If desired, may increase to 2ml IMDM and 2ml freezing media to store in 4 vials. Also, good idea to count cells and record on vial prior to freezing cells.

Cell line characteristics:

Indolent - MOC1: less aggressive based on in vivo studies. If passing 1:12 from 80% confluent T150, takes 2-4 days to reach 80% confluence. MOC1 cell lines take longer to come off the flask when being harvested compared to more aggressive cell lines.

Aggressive - MOC2: more aggressive based on in vivo studies. If passing 1:12 from 80% confluent

T150, takes 2-4 days to reach 80% confluence. Aggressive cell lines come off flask much easier compared to indolent lines.

Harvesting and passing cells from T150, 80% confluence:

1. Pour media from T150 into dump flask

2. Wash once with 10-20ml PBS. Pour out PBS wash.

3. Add 1.5ml 0.05% trypsin, tip flask to make sure trypsin covers the entire surface area and thus touches all the cells (do this quickly so that cells are not exposed to trypsin for too long), dump out trypsin, then reapply another 1.5ml of 0.25% trypsin.

4. Place in 37C incubator. Incubate for 3-4 minutes for aggressive cell lines and could take up to 10-12 min for indolent but check after 6-8 min.

5. Tap side of flask against palm of hand deliberately several times to loosen cells

6. Check under microscope to see if cells are floating freely in media. If most are not, place back in 37C incubator for 3-5 more minutes. Try not to let cells sit in trypsin for too long as this will kill the cells.

7. Once all or most of cells are floating, add 10ml of IMDM MOC line media to

neutralize the reaction.

8. Pipette media and cells from flask into a 15ml conical to pellet cells. Centrifuge at 1000 RPM x 5 min.

9. Pour out the supernatant.

10. To pass cells at 1:12 - resuspend cells in another 12 ml of media.

11. Take 1ml from this and place in new T150 flask with total volume of 22ml of IMDM MOC line media (1:12 dilution)

12. Place back into 37C incubator to grow. Should reach 80% confluence in 2-4 days.

Injection into flank of mice (heterotopic):

Cell concentration needed:

MOC1, MOC22: (inject 1e6 cells in 0.15ml) = 6.66e6 cells/ml MOC2: (inject 1e5 cells in 0.15ml) = 6.66e5 cell/ml

1. Harvest cells with 0.25% trypsin as noted above.

2. After neutralizing trypsin with IMDM MOC line media, spin down cell into pellet (1000 RPM x5 min) in 50ml conical. (Note: use 50ml conical to allow small gauge needle draw up cells for

injection.

3. Wash cells by resuspending cell pellet in 10ml of ice cold PBS (making sure to remove as much media containing FCS as possible), spin down cell into pellet again (1000 RPM x5 min)

4. Wash cells again by resuspending cell pellet in 3-6 ml of ice cold PBS (volume is

determined by size of pellet, as you will use this volume to count cells)

5. Count cellsperml using hemocytometer or automated cell counter, using trypan blue to

eliminate dead cells. Using total number of cells present (cells/ml x total ml of PBS), calculate volume needed toresuspend cell pellet to achieve 6.66e6 (MOC1, MOC22) or 6.66e5 cell/ml (MOC2) concentration.

Example, for MOC1, cell count is: 2.8e6 cells/ml x 5ml (PBS) = 14e6 cells total. 14e6 cells / 6.66e6 cell/ml = 2.1ml of PBS to suspend cell pellet in.

6. Spin down cells into pellet again. Pour out PBS supernatant (without aspiration with pipette). Resuspend pellet in calculated volume of ice cold PBS needed to reach appropriate

concentration, bearing in mind that there will be ~200ul left in the 50ml conical after pouring supernatant.

7. Transfer 50ml conical in ice and inject 0.15ml (150ul) of cells per mouse in subcutaneous flank.

8. Inject mice per standard protocol. We use 1ml syringe. We draw up cells using 1.5 inch 21 gauge needle and switch needle to ½ inch 26 gauge needle to inject.

Protocol for 1L media

Make media 2:1 IMDM to nutrient mixture

1.Thaw FBS and Penn/strep at 37C

2.To 1L filter flask add 626ml IMDM and 313ml nutrient mixture

3.Add 50ml FCS

4. Add 10ml Penn Strep

5.Filter

6.Add 1ml of 5mg/ml Insulin (or 500ul at 10mg/ml)

To make insulin – dilute 50mg of powder with 10ml sterile H20, add 50ul sterile 1N HCl, store at 4C wrapped in foil.

7. Add 40ug of Hydrocortisone

To make Hydrocoritsone - dilute 1mg of Hydrocortisone powder from Sigma into 19ml serum-free IMDM and 1ml 100% EtOH to make stock of 20ml. Use 800ul of this per Liter. Store aliquots at -20.

8.Add 5ug EGF

To make EGF - make 1ug/ul stock diluted in serum-free IMDM and add 5ul per Liter. Store aliquots at -80.

SCIENTIFIC REAGENT CATALOG NUMBERS

IMDM: sh30228.02

Hams Nutrient Mixture F10-F12 : sh30026.01

Fetal Bovine Serum, Characterized – HYCLONE: Catalog # sh30071.03, Lot #: AWK24001 Penn Strep: BW17-603E

Filter using 1L filter flask: 09-761-108

SIGMA ALDRICH REAGENT CATALOG NUMBERS

Insulin: I6634-50mg

Hydrocortisone: H0135-1mg

EMD MILLIPORE REAGENT CATALOG NUMBERS

Epidermal Growth Factor (EGF), human recombinant: 01-107