MURINE L-WRN CONDITIONED MEDIUM PROTOCOL

Taniguchi Lab
Modified from HPW Lab
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

*This protocol makes ~3L of 50% CM (for 1L use 10 plates/flasks)
*Long-term storage of CM should be at -20C or -80C
*CM can be kept at +4C for up to 2-3 weeks.

Materials

T-150 Flasks or 15-cm^2 (p150) plates		
T-175 3-layer Flasks		
HBSS no Ca2+, no Mg2+, no phenol red (Life Technologies 14175-079)	+4C	1 L
TrypLE™ Express (Life Technologies 12605-010)	room	100 ml
EDTA (SIGMA E7889-100ml)	room	100 ml
DMSO Hybri-Max (SIGMA D2650-100ml)	room	100 ml
G418 (SIGMA G8168-10ml) - 50mg/ml		
Hygrogold/Hygromycin (InvivoGen ant-hg-1) - 100mg/ml		
L-cell Medium	+4C	
DMEM high glucose (SIGMA D6429-500ml)	+4C	500 ml
100x Penicillin/Streptomycin (SIGMA P4333-100ml)	+4C	5ml
FBS (Heat inactivated)	-20C	50 ml
Primary Culture Medium	+4C	
*Make fresh weekly		
Advanced DEM/F12 (Invitrogen 12634-010)	+4C	500 ml
100x L-glutamine (SIGMA G7513-100ml)	-20C	6.25 ml
100x Penicillin/Streptomycin (SIGMA P4333-100ml)	+4C	6.25 ml
FBS (Heat inactivated)	-20C	125 ml

Calculations

- Need 25 ml (+ 5 ml due to evaporation) of L-cell Medium per T-150 flask
- Need 75 ml (+ 5 ml due to evaporation) of Primary culture Medium per T-175 3-tier flask

L-WRN Cell Culture

1. Make the L-cell medium.
2. Thaw a cryotube of L-WRN cells in the 37C water bath.
3. Add 5 ml of L-cell medium and the L-WRN cell suspension to a 15ml conical tube, and centrifuge at 700-1,000 rpm +4C for 3-5 minutes.
4. Add 30 ml of L-cell medium to a 50ml conical tube and leave on ice.
5. Aspirate supernatant, leaving ~500ul, and transfer to the 50ml tube with previously added L-cell medium.
6. Add 150 ul of Hygromycin for a final concentration of 500ug/ml, and 300ul of G418 for a final concentration of 500ug/ml.
7. Transfer the cell solution to a T-150 Flask and incubate at 37C until ~90% confluent. Change media every 2-3 days if not yet confluent.

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8. Split cells 1:5 by washing with 20 ml of PBS, aspirating, digesting with 3 ml of TrypLE for 5 min in 37C incubator, and neutralizing with 9 ml of L-cell media, followed by centrifuging for 5 min at 1,000 rpm at +4C.
9. When ~100% confluent, split cells 1:6 (5 T-150 flasks into 10 T-175 3-tier flasks).

Harvesting Conditioned Medium (CM)

1. Wash confluent cells with 50 ml PBS in each flask and aspirate twice.
2. Wash cells with 25 ml washing medium in each flask and aspirate.
3. Add 75 ml (80 ml b/c of evaporation) of **Primary cell culture medium** to each flask.
4. Incubate for 3 days at 37C.
5. Recover CM into 50 ml tubes and spin at 3000 rpm for 5 min and collect supernatant into a 500 ml bottle, leaving behind the bottom ~5 ml. Store at +4C (1^{st} CM).
6. Add fresh 75ml (80 ml b/c of evaporation) of **Primary cell culture medium** to each flask and incubate for 3 days at 37C.
7. Recover 2^{nd} CM into 50 ml tubes and spin at 3000 rpm for 5 min and collect supernatant, leaving behind the bottom ~5 ml. Combine with 1^{st} CM.
8. Add an equal volume of **Primary cell culture medium** (final 50% CM).
9. Vacuum filter all the 50% CM using a 40 um strainer.
10. Aliquot into 15 ml and/or 50 ml tubes (do not exceed 13 ml and 40 ml due to expansion) and store at -20C.

*Thaw aliquots in refrigerator overnight prior to use (keep 2-3 weeks at +4C).