

Preparation of L-Cell Conditioned Media

Cindy Rohde, 7/21/04

1. Thaw L-cells in a T25 flask.
2. Allow cells to grow to confluence, approximately 3 days.
3. Seed 1.25×10^5 cells into several T75 flasks.
4. Bring total volume of media in flask to 30 mL.
5. Allow cells to incubate for 1-1.5 weeks.
6. Remove media from cells. (If desired, can add another 30 mL to cells, incubate cells for another 1-1.5 weeks and harvest conditioned media. Can repeat this process 2-3 times.)
7. Spin down L-cell conditioned media at 2000 rpm for 20 min.
8. Filter media to remove cellular debris.
9. Aliquot out media and store at -20°C until needed.

Use 30% L-cell conditioned media for BMMs.

L-Cell Media

MEM

15%FBS

non-essential amino acids (100x stock)

sodium pyruvate (100x stock)

pen/strep (100x stock)