TECHNICAL MANUAL

Mouse Long-Term Culture-Initiating Cell (LTC-IC) Assays

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

Table of Contents

1.0 Introduction

The long-term culture (LTC) system was developed to detect and count primitive hematopoietic progenitor cells.[1-4] In this sytem, primitive hematopoietic cells associate with an adherent stromal layer and are able to generate myeloid clonogenic progenitor cells and mature granulocytes and macrophages for many weeks if provided with appropriate medium, supplements, and culture conditions. LTC systems have also been developed for generation and quantitation of lymphoid cells.[5]

The primary application of this culture system is the quantification of primitive hematopoietic progenitor cells capable of initiating and sustaining myelopoiesis for several weeks in vitro. These cells share functional and phenotypic properties with in vivo repopulating stem cells and are commonly referred to as long-term culture-initiating cells (LTC-IC).

The unique features of the long-term culture system have allowed the development of the LTC-IC assay to detect and quantitate primitive hematopoietic cells which share phenotypic and functional properties with in vivo repopulating stem cells.[6] In mouse long-term cultures, colony-forming units (CFU) detected after ≥ 4 weeks represent the progeny of LTC-IC since CFU present in the input cell suspension have undergone terminal differentiation by this time.

Quantitation of LTC-IC in a test cell suspension requires culturing the cells on a supportive feeder layer of irradiated marrow cells or suitable mouse fibroblast cell lines such as AFT024.[7] Limiting dilution analysis[8] is used to determine the frequency of LTC-IC as well as the average number of CFU produced per LTC-IC. Once the average number of CFU per LTC-IC is established, the LTC-IC content of a sample can be determined by a bulk culture LTC-IC assay, provided that the same source of test cells is used and the assay conditions are identical. The LTC-IC content is then calculated by dividing the total output of CFU by the average number of CFU produced per LTC-IC.

2.0 Glossary of Terms Used

ABBREVIATION	DESCRIPTION
LTC	Long-term culture
LTC-IC	Long-term culture-initiating cell
LTCM	Long-term culture medium
FBS	Fetal bovine serum
IMDM	Iscove's Modified Dulbecco's Medium
CFU	Colony-forming unit
D-PBS	Dulbecco's phosphate-buffered saline
BM	Bone marrow

3.0 Equipment and Materials Required

3.1 Equipment

- Biohazard safety cabinet certified for Level II handling of biological materials

  Note: All procedures for cell processing and setup of CFU assays should be performed using sterile technique and universal handling precautions.
- Incubator set at 37°C with 5% CO_2 in air and ≥ 95% humidity

  Note: Use of water-jacketed incubators with a water pan placed in the chamber is recommended. It is important to use medical grade CO_2 as inhibition of CFU growth due to toxic substances present in the CO_2 gas source has been reported.
- Inverted microscope for colony counting
- Use of a quality inverted microscope equipped with a 10X or 12.5X eyepiece objective, 2X, 4X, and 10X planar objectives and a blue filter is recommended
- Standard light microscope for cell counting
- Laboratory centrifuge
- Vortex
- Pipette-aid
- Micropipettors
- Multi-channel pipettor
- Automated cell counter or Neubauer hemocytometer

3.2 Materials

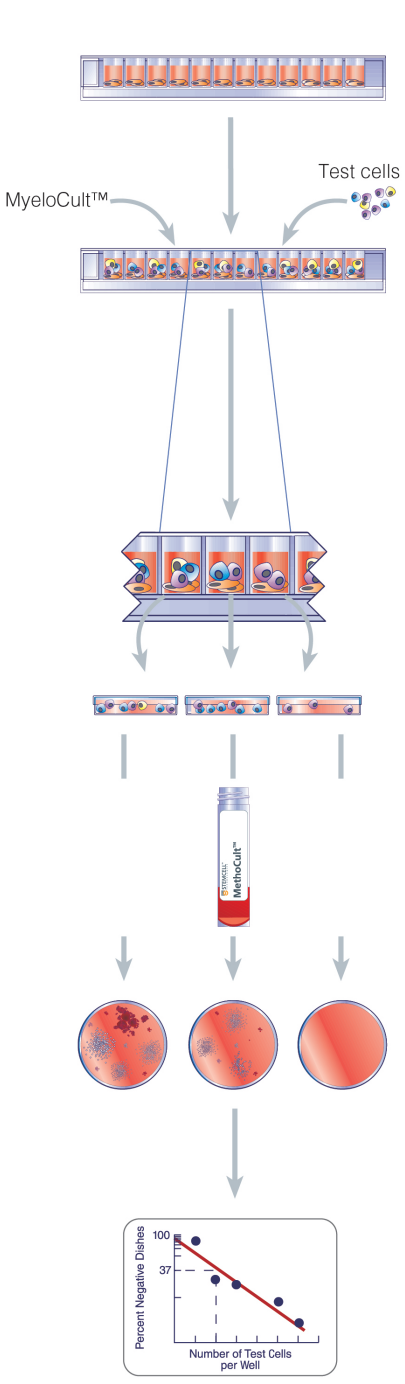
- Sterile serological pipettes: 2 mL (Catalog #38002), 5 mL (Catalog #38003)
- Sterile polystyrene tubes: 5 mL (12 x 75 mm; Catalog #38007), 14 mL (17 x 95 mm; Catalog #38008)
- Conical tubes: 15 mL (Catalog #38009), 50 mL (Catalog #38010)
- Sterile pipette tips
- Syringes (luer lock): 3 mL (Catalog #28230), 6 mL, 12 mL
- 16 Gauge Blunt-End Needles (Catalog #28110)
- 35 mm Culture Dishes (Catalog #27100) or SmartDish™ 6-well culture plates (Catalog #27370)
- 100 mm culture dishes (e.g. Treated Tissue Culture Dishes, Catalog #27125)
- 245 mm square dishes (e.g. Corning® 245 mm Square Dish, Non-Treated, Catalog #38020) or 150 mm culture dishes
- 60 mm Gridded Scoring Dishes (Catalog #100-0085) or STEMgrid™-6 counting grid (Catalog #27000)
- Permanent fine-tip marker
- 96-Well Treated Tissue Culture Plate (e.g. Catalog #27136)

3.3 Additional Materials and Reagents

PRODUCT	CATALOG #
MyeloCult™ H5100	05150
MethoCult™ GF M3434	03434
3% Acetic Acid with Methylene Blue	07060
Alpha MEM with Nucleosides	36450
Hydrocortisone	74142
RPMI 1640 Medium	36750
HBSS, Modified (Without Ca++ and Mg++)	37250
Iscove's MDM with 2% FBS	07700
Trypsin in Citrate Saline (0.25%)	07400
Trypsin-EDTA (0.25%)	07901
Collagen Solution	04902
EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit	19856
D-PBS (Without Ca++ and Mg++) (PBS)	37350
L-Calc™ Limiting Dilution Software	28600

For a complete list of products available from STEMCELL Technologies Inc., visit www.stemcell.com.

4.0 LTC-IC Assay Procedure Diagram



1

Establish Feeders
Irradiated marrow cells

2

Add Serial Dilutions of Test Cells

3

Maintain Cultures
For ≥ 4 weeks with weekly 1/2 medium changes

4

Harvest and Plate
Harvest all cells from each well, including adherent and non-adherent cells, and transfer into individual methylcellulose cultures. Incubate for 12 days.

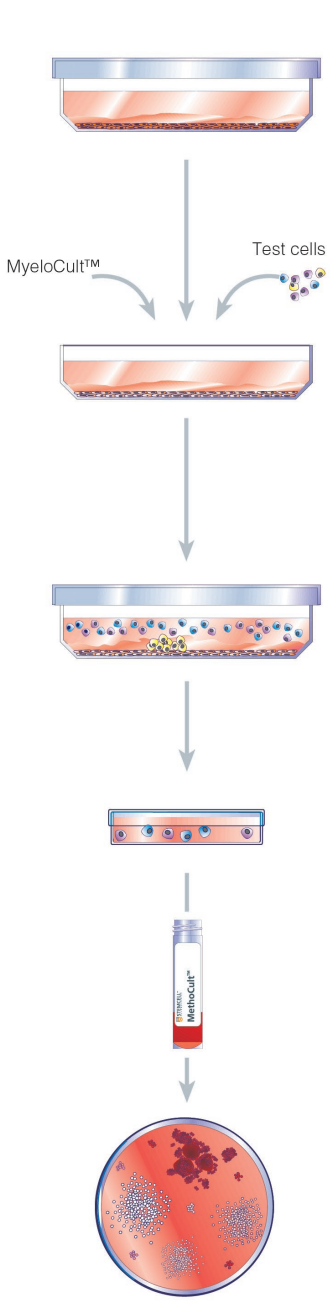
5

Score Dishes
Record growth (≥1 colony) or no growth (no colonies)

6

Calculate LTC-IC Frequency
Use a statistical software program for limiting dilution analysis, e.g. L-Calc™

5.0 Bulk LTC-IC Assay Procedure Diagram



1
Establish Feeders
Irradiated marrow cells

2
Add Known Number of Test Cells

3
Maintain Cultures
For ≥ 4 weeks with weekly 1/2 medium changes

4
Harvest and Plate
Harvest entire dish, including adherent and non-adherent cells, and plate appropriate aliquot in methylcellulose cultures. Incubate for 12 days.

5
Count Total Number of Colonies
Calculate number of LTC-IC in test cell suspension based on the average output of CFU per LTC-IC as determined by a limiting dilution assay

6.0 Isolating Mouse Bone Marrow (BM) Cells

Ensure that animals are sacrificed using procedures approved by your institution.

1. Wet the pelt thoroughly with 70% isopropyl alcohol, then clip and peel back to expose hind limbs. Using sterile sharp scissors (to avoid splitting of the bone), cut the knee joint in the center and remove ligaments and excess tissue.
2. Trim the ends of the long bones to expose the interior of the marrow shaft.
3. Collect the marrow cells in 1 - 2 mL of Alpha MEM with Nucleosides containing 2% fetal bovine serum (FBS). Flush the marrow from the femoral shaft using a 21 gauge needle attached to a 3 mL syringe. A smaller needle (22 or 23 gauge) may be required to remove cells from the tibia. Use the same medium to flush the bones of 1 - 3 animals.
4. Prepare a single-cell suspension by gently aspirating several times using the same needle and syringe.
5. Store the cells on ice.

   Note: For most applications, including LTC-IC assays, it is not necessary to perform density gradient centrifugation or to wash the cells in fresh buffer.
6. To count nucleated cells, remove a small aliquot of cell suspension and dilute 1 in 50 to 1 in 100 in 3% Acetic Acid with Methylene Blue. Count nucleated cells using a hemocytometer.

Expected recovery of nucleated cells: $1 - 2 \times 10^7$ cells per femur
$0.6 - 1.2 \times 10^7$ cells per tibia
5×10^7 cells per mouse (2 femur, 2 tibia)

7.0 Long-Term Culture Without Pre-Established Feeders

7.1 Preparation of Long-Term Culture Medium (LTCM)

Use sterile technique to prepare LTCM (MyeloCult™ H5100 + Hydrocortisone).

1. Thaw MyeloCult™ H5100 in the refrigerator (2 - 8°C), at room temperature (15 - 25°C), or at 37°C until just thawed. Mix well by swirling.

   Note: If not used immediately, store at 2 - 8°C for up to 1 month.
2. Prepare a stock solution of Hydrocortisone by dissolving Hydrocortisone powder in Alpha MEM with Nucleosides to yield a concentration of 1×10^{-3} M. Filter sterilize using a 0.2 µm low protein binding syringe filter.

   Note: Prepare fresh Hydrocortisone stock solution each week.
3. Prepare a 1 in 10 dilution of the 1×10^{-3} M Hydrocortisone stock solution to yield 1×10^{-4} M. Dilute this 1×10^{-4} M working stock solution 1 in 100 in MyeloCult™ H5100 to yield a final Hydrocortisone concentration of 1×10^{-6} M (e.g. 1 mL of 1×10^{-4} M Hydrocortisone in 99 mL MyeloCult™ H5100).

   Note: If not used immediately, store LTCM at 2 - 8°C for up to 1 week.

7.2 Initiation of Cultures

1. Resuspend marrow cells in LTCM (MyeloCult™ H5100 with 10^{-6} M hydrocortisone) to the appropriate cell density and place in tissue culture dishes or flat-bottom culture plates (Table 1).

   Note: For long-term culture and preparation of feeder layers for LTC-IC, initiate cultures with ~1 - 1.5 x 10^6 BM cells per cm^2 culture surface area.

Table 1. Recommended Plating Densities

VOLUME (mL)	TYPE OF CULTUREWARE	CELL DENSITY
8	60 mm tissue culture dishes	3 x 10^7 cells/dish
8	T-25 cm^2 flasks	3 x 10^7 cells/flask
2	35 mm tissue culture dishes	7.5 x 10^6 cells/dish
1	24-well flat-bottom plate	2 x 10^6 cells/well
0.15	96-well flat-bottom plate	3 x 10^5 cells/well

2. Place 35 mm dishes or 60 mm dishes inside a covered 100 mm Petri dish with an additional uncovered 35 mm dish containing 4 mL sterile distilled water. Place 96-well plates in a suitable covered container (e.g. covered 245 mm square dishes) containing uncovered 35 mm dishes with sterile water.
3. Incubate cultures at **33°C** and 5% CO_2 with ≥ 95% humidity for ≥ 4 weeks.

7.3 Medium Changes

Perform half-medium changes weekly with fresh LTCM, as below:

For culture dishes:

1. Gently rock the culture to loosen non-adherent cells.
2. Using a sterile 2 mL serological pipette, draw up all the LTCM and cells and return one-half of the original volume to the dish. Discard the remainder of the medium.

   Note: Due to evaporation, the total volume may be less than in the original culture.
3. Add one-half of the original volume of fresh LTCM. Incubate at **33°C** and 5% CO_2 with ≥ 95% humidity.

   Note: Do not disturb the adherent layer; the ends of pipette tips should not contact the adherent layer. Add fresh LTCM dropwise to avoid disturbing the adherent layer. To avoid contamination, care must be taken not to touch tips on the exterior of the wells. New tips must be used each time cells and/or medium are removed from wells.

   Example: 2 mL cultures in 35 mm tissue culture dish: Remove all medium (1.85 mL) with a sterile 2 mL serological pipette. Return 1 mL of medium containing cells to the dish and discard* the remaining 0.85 mL. Add 1 mL fresh LTCM.

   ** If desired, cells can be used to measure CFU output in non-adherent fraction at different timepoints by replating cells in MethoCult™ GF M3434 (section 7.5).*
4. Proceed to section 7.4 for a description of long-term cultures during the culture period.

7.4 Appearance of Long-Term Cultures

Following 1 week of incubation, an adherent layer comprising endothelial cells, fibroblasts, and adipocytes should be partially established (although not confluent). After 2 weeks of incubation, the feeder/stromal layer should be greater than 70 - 80% confluent and areas of the cultures may contain clusters of hematopoietic cells with a cobblestone-like appearance. Long-term cultures using normal BM cells should continue to produce clonogenic cells in the supernatant for 12 weeks or longer.

Following the desired incubation period, (typically ≥ 4 weeks), proceed to section 7.5 for harvesting cells.

7.5 Harvesting Long-Term Cultures

Harvest long-term cultures (both adherent and non-adherent cells) and assay for clonogenic progenitor cells in methylcellulose-based medium.

The following example is for harvesting cultures from 35 mm dishes.

1. Pipette non-adherent cells and medium from the tissue culture dish into a sterile 15 mL conical tube (the “harvest tube”).
2. Rinse the adherent layer with 1 mL HBSS Without Ca++ and Mg++, gently swirl plate and add all medium to the harvest tube.
3. Add 1.0 mL of Trypsin in Citrate Saline (0.25%) or Trypsin-EDTA (0.25%) and incubate at 37°C with 5% CO_2 in air and ≥ 95% humidity for approximately 1 - 2 minutes (maximum 10 minutes). At intervals, swirl culture gently and examine using an inverted microscope for evidence of detachment of the adherent layer. Once adherent layer has started to detach, add 0.2 mL FBS to neutralize the trypsin.
4. Using a sterile pipette, repeatedly pipette the trypsin solution over the surface of the dish to ensure all adherent cells are detached and to make a single-cell suspension. Add all cells and medium to the harvest tube.
5. Perform a second trypsinization if significant numbers of adherent cells are still present (examine cultures using inverted microscope).
6. Rinse the culture dish twice with Iscove’s MDM with 2% FBS. Add all cells and medium to the harvest tube.
7. Fill harvest tube with Iscove’s MDM with 2% FBS and centrifuge at 300 x *g* for 7 - 10 minutes.
8. Remove supernatant and discard. Resuspend cells in 0.5 - 1 mL Iscove’s MDM with 2% FBS. Record volume and perform a nucleated cell count using 3% Acetic Acid with Methylene Blue and a hemocytometer.
9. Plate an appropriate number of cells in MethoCult™ GF M3434.

   Note: For complete instructions, refer to the Technical Manual: Mouse Colony-Forming Unit Assays using MethoCult™ (Document #10000005597), available at www.stemcell.com or contact us to request a copy.
10. Incubate cultures at 37°C and 5% CO_2 with ≥ 95% humidity for 12 - 14 days.
11. Count and record the total number of colonies per dish.

8.0 LTC-IC Assay of Mouse Cells Using Primary Marrow Feeders and Limiting Dilution Analysis

8.1 Establishment of Marrow Feeder Layers

1. Isolate mouse bone marrow cells as described in section 4.0.
2. Resuspend marrow cells in LTCM (see section 7.1). Plate at recommended cell densities in tissue cultureware as indicated in Table 1 (section 7.2).

   Note: 96-well plates are recommended, to allow for multiple conditions/higher throughput required for limiting dilution experiments.
3. Perform weekly half-medium changes as described in section 7.3.
4. Incubate cultures at **33°C** and 5% CO_2 with ≥ 95% humidity for approximately 2 - 3 weeks or until an adherent layer has established and is about 80% confluent.
5. Inactivate hematopoietic progenitor cells within the feeder/stromal layer by irradiation. Irradiate the feeder layer with 1500 cGy using a Cesium-137 or X-ray source.

   Note: The feeder layer may be used within 10 - 14 days following irradiation. Irradiated feeder layers should have weekly half-medium changes (see section 7.3) until use.

   Note: Stromal cell lines such as AFT024 may be used as an alternative to primary marrow feeders.[9,10]
6. Proceed to section 8.2 for adding test cells.

8.2 Adding Test Cells to Irradiated Feeder Layer

1. Remove 0.1 mL of LTCM from the wells of the 96-well plate using a multi-channel pipettor and sterile tips and discard (approximately 50 µL of LTCM should remain in each well).
2. Resuspend the test cell suspension of unseparated or purified mouse cells in the appropriate volume of LTCM and add to test wells in 0.1 mL. Refer to Table 2 for recommended plating densities.

Note: Optimal plating concentrations may vary depending on mouse strains and the hematopoietic stem and progenitor cell enrichment procedure used and should be established in each laboratory.

Table 2. Recommended Plating Densities on Irradiated Feeder Layers

CELL SOURCE*	RECOMMENDED PLATING DENSITY (cells/well of 96-well plate)**
Normal mouse bone marrow, unprocessed†	3×10^4
	1.5×10^4
	7.5×10^3
Normal mouse bone marrow enriched by depletion of lineage positive cells using EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit	3000
	1500
	750
Normal mouse bone marrow, further enriched for Sca1+/Lin- cells	300
	150
	75
	37.5

* Cell densities are given for cells isolated from normal C57/Bl6 mice.
** Plating 12 - 16 wells for each sample is recommended.
† Unprocessed refers to cells processed only as outlined for isolation of the cells, as described in section 6.0.

3. Incubate cultures at **33°C** and 5% CO_2 with ≥ 95% humidity for 4 weeks with weekly half-medium changes (see section 7.3).
4. Proceed to section 8.3 for harvesting cells.

8.3 Harvesting Cells From Limiting Dilution Cultures

1. Remove all LTCM and non-adherent cells to 5 mL sterile polystyrene tubes using a pipettor and sterile tips.

   Note: A multi-channel pipettor can be used to sterilely harvest 3 wells at a time. Test tube rack should allow uncapped test tubes to sit tightly together. To avoid contamination, care must be taken not to touch tips on the exterior of the wells. New tips must be used each time cells and/or medium is removed from wells.
2. Rinse each well once with 0.1 mL HBSS, Modified (Without Ca++ and Mg++) and add to appropriate tube.
3. Add 0.1 mL of Trypsin-EDTA (0.25%) or Trypsin in Citrate Saline (0.25%) and incubate at 37°C and 5% CO_2 with ≥ 95% humidity for approximately 1 - 2 minutes (maximum 10 minutes). At intervals, swirl the culture gently and examine using an inverted microscope for evidence of detachment of the adherent layer. If desired, add 10 µL of FBS to neutralize trypsin.
4. Resuspend both adherent and non-adherent cells with micropipettor and sterile tips or multichannel pipettor with sterile tips to obtain a single-cell suspension. Add cells and medium to the tube.
5. Rinse each well once with Iscove's MDM with 2% FBS and add to tube.
6. Fill tube with Iscove's MDM with 2% FBS and centrifuge at 300 x *g* for 7 - 10 minutes. Remove supernatant without disturbing cell pellet, leaving 0.1 mL of medium in the tube.
7. Vortex tube and add 1 mL of methylcellulose medium (e.g. MethoCult™ GF M3434) using a 3 mL syringe and 16 gauge needle. Vortex and let stand for at least 5 minutes to allow bubbles to rise to the top.
8. Plate each tube (contents of one well) individually onto a 35 mm dish using a 1 mL syringe (without needle attached).
9. Incubate at 37°C and 5% CO_2 with ≥ 95% humidity for 12 - 14 days.
10. Count colonies.

    Note: A well is scored as positive if one or more colonies are detected, or scored as negative if no colonies are present. For further information on counting colonies, refer to the Technical Manual: Mouse Colony-Forming Unit Assays using MethoCult™ (Document #10000005597), available at www.stemcell.com or contact us to request a copy.
11. The LTC-IC frequency in the test cell population is calculated from the proportion of negative wells (no CFU present) and the method of maximum likelihood.[16] Statistical analysis can be performed using L-Calc™ Limiting Dilution Software.

Table 3. Frequency of LTC-IC in Mouse (C57Bl/6 Strain) Cell Populations

SOURCE	FREQUENCY	REFERENCE
Adult bone marrow	0.001 - 0.005%	Lemieux et al. (1995) Miller et al. (1996) Kotsianidis et al. (2006) Lymperi et al. (2011)
Lin^- $CD45^+AnV^-$ Adult bone marrow	~ 1%	Jang and Sharkis (2007)
$Lin^-Sca1^+Kit^+$ Adult bone marrow	~ 5% (0.5 - 10%)	Lancini et al. (2014)
$CD45^+EPCR^+CD48^-CD150^+$	32 - 52%	Kent et al. (2009)
$CD45^+EPCR^+CD48^-CD150^-$	3.5 - 8.5%	Kent et al. (2009)

9.0 Quantitation of LTC-IC Using Bulk Cultures

9.1 Initiation of Bulk Cultures

Refer to section 5.0 for the bulk culture LTC-IC procedure diagram.

1. Establish feeder layers as described in section 8.1.
2. Resuspend test cells in the required volume of LTCM. Refer to Table 4 for the recommended volumes and cell densities for various types of cultureware. Gently remove the medium from tissue culture dishes containing irradiated feeder cells (section 8.1) and replace with LTCM containing test cells.

Table 4. Recommended Cell Densities for Initiation of LTC-IC on Primary Marrow Feeders

VOLUME (mL)	TYPE OF CULTUREWARE	CELL DENSITY*
8.0	60 mm tissue culture dishes	3.0×10^6 cells/dish
8.0	T-25 cm^2 flasks	3.0×10^6 cells/flask
2.0	35 mm tissue culture dishes	7.5×10^5 cells/dish
1.0	24-well plate	2×10^5 cells/well
0.15	96-well flat-bottom plate	3.0×10^4 cells/well

* Unprocessed BM cells from normal CS7/BL6 mice

3. Place 35 mm dishes or 60 mm dishes inside a covered 100 mm Petri dish with an additional uncovered 35 mm dish containing 4 mL sterile distilled water. Place 24-well or 96-well plates in a suitable covered container (e.g. covered 245 mm square dishes) containing uncovered 35 mm dishes with sterile water.
4. Incubate cultures at **33°C** and 5% CO_2 with ≥ 95% humidity for ≥ 4 weeks.

9.2 Medium Changes

1. Perform half-medium changes weekly with fresh LTCM, as below:

   For culture dishes:

   a) Gently rock the culture to loosen non-adherent cells.

   b) Using a sterile 2 mL serological pipette, draw up all the LTCM and cells and return one-half of the original volume to the dish. Discard the remainder of the medium.

   Note: Due to evaporation, the total volume may be less than present in the original culture.

   c) Add one-half of the original volume of fresh LTCM.

   Note: Do not disturb the adherent layer; the ends of pipette tips should not contact the adherent layer. Add fresh LTCM dropwise to avoid disturbing the adherent layer. To avoid contamination, care must be taken not to touch tips on the exterior of the wells. New tips must be used each time cells and/or medium are removed from wells.

   Example: 2 mL cultures in 35 mm tissue culture dish: Remove all medium (1.85 mL) with a sterile 2 mL serological pipette. Replace 1 mL of medium containing cells and discard* the remaining 0.85 mL. Add 1 mL fresh LTCM.

   ** If desired, cells can be used to measure CFU output in non-adherent fraction at different timepoints by replating cells in MethoCult™ GF M3434 (section 7.5).*

2. Proceed to section 9.3 for harvesting cells.

9.3 Harvesting Cells from Bulk Cultures

Following the desired incubation period (typically ≥ 4 weeks), harvest LTC-IC cultures (both adherent and non-adherent cells) and assay for clonogenic progenitor cells in methylcellulose-based medium.

The following example is for harvesting cells from 35 mm dishes.

1. Pipette non-adherent cells and medium from the tissue culture dish into a sterile 15 mL conical tube (the "harvest tube").
2. Rinse the adherent layer with 1 mL HBSS, Modified (Without Ca++ and Mg++), gently swirl plate and add all medium to the harvest tube.
3. Add 1.0 mL of Trypsin in Citrate Saline (0.25%) or Trypsin-EDTA (0.25%) and incubate at 37°C with 5% CO_2 in air and ≥ 95% humidity for approximately 1 - 2 minutes (maximum 10 minutes). At intervals, swirl culture gently and examine using an inverted microscope for evidence of detachment of the adherent layer. Once the adherent layer has started to detach, add 0.2 mL of FBS to neutralize the trypsin.
4. Using a sterile pipette, repeatedly pipette the trypsin solution over the surface of the dish to ensure all adherent cells are detached and to make a single-cell suspension. Add all cells and medium to the harvest tube.
5. Perform a second trypsinization if significant numbers of adherent cells are still present (examine cultures using inverted microscope).
6. Rinse the culture dish twice with Iscove's MDM with 2% FBS. Add all cells and medium to the harvest tube.
7. Fill the harvest tube with Iscove's MDM with 2% FBS and centrifuge at 300 x *g* for 7 - 10 minutes.
8. Remove the supernatant and discard. Resuspend the cells in 0.5 - 1 mL of Iscove's MDM with 2% FBS. Record the volume and perform a nucleated cell count using 3% Acetic Acid with Methylene Blue and a hemocytometer.
9. Plate an appropriate number of cells in MethoCult™ GF M3434.

   Note: For complete instructions, refer to the Technical Manual: Mouse Colony-Forming Unit Assays using MethoCult™ (Document #10000005597), available at www.stemcell.com or contact us to request a copy.
10. Incubate cultures at 37°C and 5% CO_2 with ≥ 95% humidity for 12 - 14 days.
11. Count and record the total number of colonies per dish. Calculate the total number of LTC-IC-derived CFU.

10.0 References

1. Dexter TM et al. (1977) Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol 91(3): 335–44.
2. Dexter TM et al. (1980) The role of cells and their products in the regulation of in vitro stem cell proliferation and granulocyte development. J Supramol Struct 13(4): 513–24.
3. Ploemacher RE et al. (1989) An in vitro limiting-dilution assay of long-term repopulating hematopoietic stem cells in the mouse. Blood 74(8): 2755–63.
4. Wineman J et al. (1996) Functional heterogeneity of the hematopoietic microenvironment: rare stromal elements maintain long-term repopulating stem cells. Blood 87(10): 4082–90.
5. Whitlock CA & Witte ON. (1982) Long-term culture of B lymphocytes and their precursors from murine bone marrow. Proc Natl Acad Sci USA 79(11): 3608–12.
6. Lemieux ME et al. (1995) Characterization and purification of a primitive hematopoietic cell type in adult mouse marrow capable of lymphomyeloid differentiation in long-term marrow “switch” cultures. Blood 86(4): 1339–47.
7. Kent DG et al. (2009) Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. Blood 113(25): 6342–50.
8. Fazekas de St Groth. (1982) The evaluation of limiting dilution assays. J Immunol Methods 49(2): R11-23.
9. Moore KA et al. (1997) In vitro maintenance of highly purified, transplantable hematopoietic stem cells. Blood 89(12): 4337–47.
10. Woehrer S et al. (2013) Long-term culture-initiating cell assay for mouse cells. In: C. Helgason & C. Miller (Eds.), Basic Cell Culture Protocols (pp. 257–66). New York, NY: Humana Press.
11. Kotsianidis I et al. (2006) Regulation of hematopoiesis in vitro and in vivo by invariant NKT cells. Blood 107(8): 3138–44.
12. Miller CL et al. (1996) Studies of W mutant mice provide evidence for alternate mechanisms capable of activating hematopoietic stem cells. Exp Hematol 24(2): 185–94.
13. Lymperi S et al. (2011) Inhibition of osteoclast function reduces hematopoietic stem cell numbers in vivo. Blood 117(5): 1540–9.
14. Jang Y-Y & Sharkis SJ. (2007) A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood 110(8): 3056–63.
15. Lancini C et al. (2014) Tight regulation of ubiquitin-mediated DNA damage response by USP3 preserves the functional integrity of hematopoietic stem cells. J Exp Med 211(9): 1759–77.
16. Miller CL et al. (1997) Impaired steel factor responsiveness differentially affects the detection and long-term maintenance of fetal liver hematopoietic stem cells in vivo. Blood 89(4): 1214–23.

TECHNICAL MANUAL

Mouse Long-Term Culture-Initiating Cell (LTC-IC) Assays

STEMCELL™ TECHNOLOGIES

TOLL-FREE PHONE 1 800 667 0322
PHONE +1 604 877 0713
INFO@STEMCELL.COM
TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

DOCUMENT #10000005500 VERSION 01 AUG 2022 | FOR INTERNAL USE: MATERIAL #28417