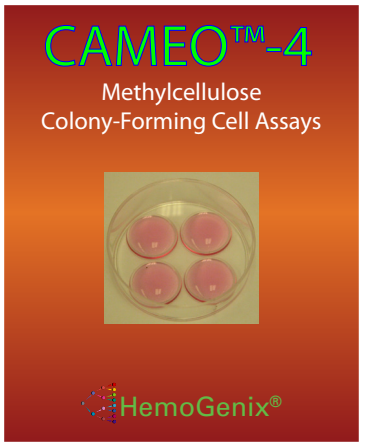
Ordering Information
Tel: (719) 264-6250
Fax: (719) 264-6253
Email: info@hemogenix.com
Order online at www.hemogenix.com

Technical Support
Tel: (719) 264-6250
Email: info@hemogenix.com

HemoGenix®, Inc
1485 Garden of the Gods Road
Suite 152
Colorado Springs, CO 80907
U.S.A.
Tel: (719) 264-6250
Fax: (719) 264-6253
Web: www.hemogenix.com

FOR *IN VITRO* RESEARCH USE ONLY (RUO)
NOT FOR CLINICAL DIAGNOSTIC USE



CAMEO™-4
Basic and Applied Research & Stem Cell Processing Laboratory Applications

Instruction Manual

(Version 4.2011)

For *In Vitro* Research Use Only.
Not for clinical diagnostic use.

HemoGenix®, Inc

TABLE OF CONTENTS

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

10. REFERENCES

1. **Bradley TR, Metcalf D**: The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med (1966), 44:287.
2. **Pluznik DH, Sachs L**: The induction of clones of normal mast cells by a substance from conditioned medium. Exp Cell Res (1966), 43:553.
3. **Stephenson JR, Axelrad AA, McLeod DL, Shreeve MM**: Induction of colonies of hemoglobin-synthesizing cells by erythropoietin in vitro. Proc Natl Acad Sci USA (1971), 44:517.
4. **Axelrad AA, McLeod DL, Shreeve MM, Heath DS**: Properties of cells that produce erythrocytic colonies in vitro. In Hemopoiesis in Culture. Edited by Robinson W. Washington D.C.: U.S. Gorvernment Printing Office; (1973) pp. 226.
5. **Iscove NN, Sieber F, Winterhalter KH**: Erythroid colony formation in cultures of mouse and human bone marrow; analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on agarose-concanavalin A. J Cell Physiol (1974), 83:309-320.
6. **Iscove NN, Sieber F**: Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture . Exp Hemat (1975), 3:32-43.
7. **Rich IN & Kubanek B**: The effect of reduced oxygen tension on colony formation of erythropoietic cells in vitro. Brit J Haematol (1982), 52:579-588.
8. **Rich IN**: A role for the macrophage in normal hemopoiesis: II. Effect of varying oxygen tensions on the release of hemopoietic growth factors from bone marrow-derived macrophages in vitro. Exptl. Hemat. (1986), 8:746-751.
9. **Musk P, Rich IN**: A new high throughput stem cell and multilineage progenitor cell assay for hemotoxicity testing. The Toxicologist (2001), 66.
10. **Rich IN, Musk P**: HALO - A multifunctional colony-forming-based assay platform for drug development and basic and clinical research. Blood (2002), 100:618a
11. **Rich IN**: In vitro hemotoxicity testing in drug development. A review of past, present and future applications. Current Opinion in Drug Discovery and Development. (2003) 6:100-109.
12. **Rich IN and Hall KM**: Validation and development of a predictive paradigm for hemotoxicology using a multifunctional bioluminescence colony-forming proliferation assay. Tox Sci (2005) 87:427-441.
13. **Crouch SPM, Kozlowski R, Slater KJ, Fletcher J**: The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicty. J Immunol Meth (2000), 160:81-88.

8. RECOMMENDATIONS AND TIPS BEFORE USING THE CAMEO™-4 ASSAY KIT CELL CULTURE MIXES.

(i) Storage and Thawing Procedures

a. Ensure that the CAMEO™-4 Master Mix is thawed prior to use.
b. An experiment or study may be staged prior to adding the cells. If the CAMEO™-4 Master Mix is dispensed into tubes, cap the tubes tightly and store at 4°C is they will be used the following day. Otherwise freeze and thaw as described on page on the day of use.
c. Store any remaining CAMEO™-4 Master Mix at 4°C.

(ii) **Dispensing the CAMEO™-4 Master Mix and the Culture Master Mix containing cells**

a. Use a positive displacement repeater pipettes for all transfers involving methylcellulose–containing medium.
b. To improve %CVs, fit the syringe of the repeater pipette with a sterile pipette tip prior to dispensing the Culture Master Mix into the wells.
c. Using normal syringes with needles will result in inaccurate dispensing and greater variation between replicate wells.

9. Troubleshooting

A. Cultures dry out

Small volume cultures tend to dry out very quickly despite a "fully humidified" incubator. If this occurs, place the culture plates either in 100mm Petri dishes containing a 35mm Petri dish without a lid with 2-3ml of sterile water. For large numbers of culture dishes, use a large container and place a beaker full of sterile water in the center of the container. This container can be glass or a plastic sandwich box. Place aluminium foil loosely over container or drill holes in the sandwich box lid to allow for gas exchange.

B. High replicate or experimental variation

This is usually caused by several factors. The first is variations caused during dispensing methylcellulose-containing medium. Methylcellulose is notoriously difficult to dispense. Do not use syringes and needles. This method is inaccurate and can lead to extremely high variations. Use a positive displacement repeater pipette to dispense all methylcellulose-containing reagents. Also ensure that all pipettes are properly calibrated. Small errors is dispensing can lead to large variations.

Ensure that cell concentration calculations have been performed properly and that volumes are correctly dispensed. Similarly, check all growth factor/cytokine doses if these are added separate and that the volumes are correctly dispensed.

Colony counting is a subjective procedure regardless of whether it is performed manually or automatically. Unfortunately, there are no standards and controls for the CFC/CFU assay and therefore the assay cannot be calibrated, standardized or validated. The resulting variations can only be used using an assay such as HALO®.

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

1. INTRODUCTION

HemoGenix® divides its cell-based assays into proliferation and differentiation assays. CAMEO™-4 is a miniaturized, methylcellulose colony-forming cell (CFC) or unit (CFU) differentiation assay that has been developed for human and animal stem cell and basic research applications, including veterinary applications. In addition, four separate CAMEO™-4 assays have also been developed for hematopoietic stem cell processing laboratories.

In 1966, Bradley and Metcalf in Melbourne, Australia and Pluznik and Sachs in Rehovot, Israel, discovered a population of cells that, when stimulated by conditioned medium or stromal cells, produced colonies of granulocytes and macrophages that could be detected morphologically under the microscope. The population of cells producing these colonies was originally designated the colony-forming unit – culture (CFU-C). This population is now generally called the granulocyte-macrophage colony-forming cell (GM-CFC) and is the progenitor of two, more mature precursor populations called the granulocyte colony-forming cell (G-CFC) and the macrophage colony-forming cells (M-CFC). The substance that stimulated the production of these colonies *in vitro* was eventually called granulocyte-macrophage colony-stimulating factor (GM-CSF). In 1971, Stevenson and colleagues discovered a colony-forming population with limited proliferative capacity that produced small erythroid colonies under the influence of erythropoietin. These were called colony-forming units-erythroid (CFU-E). Shortly thereafter, Axelrad and coworkers discovered another erythropoietic population that required higher concentrations of erythropoietin and longer incubation times and was the progenitor of the CFU-E. This population was designated the burst-forming unit – erythroid (BFU-E). Eventually, colony-forming cells for multiple stem cell populations every primary lympho-hematopoietic lineage had been discovered.

The original colony-forming assays used either agar or plasma clots to immobilize the cells so that when division occurred, the cells remained in place to form a colony. The colonies were identified by the functional ability of the primitive cells to differentiate and mature. Although the proliferation process is required to produce and expand the colonies, proliferation per se is not measured using the CFC/CFU assay, because the readout of the assay is the number of colonies containing differentiated and mature cells.

In 1972, Iscove et al found that agar could be replaced with water-soluble methyl cellulose, making the culture procedure easier to perform. In 1978, Bradley et al demonstrated that culturing GM-CFC under low oxygen tension was advantageous for growth. Similar results were found by Rich & Kubanek in 1982 for CFU-E and BFU-E using a miniaturized assay system that is now called CAMEO™-4.

CAMEO™-4 was the predecessor to the HALO Platform, the original version of which also incorporated methylcellulose. This version of HALO is now called CAMEO™-96.

2. INTENDED USE

CAMEO™-4 is a miniaturized, *in vitro* colony-forming cell (CFC) or unit (CFU) assay that detects the differentiation ability and/or potential of lympho-hematopoietic stem, progenitor or precursor cells to form colonies in semi-solid methylcellulose.

CAMEO™-4 Applications

A. All basic, applied and veterinary research applications where lympho-hematopoietic cell differentiation ability and/or potential is required.
B. Hematopoietic stem cell transplantation and cord blood bank processing laboratories.

A. CAMEO™-4 for Basic and Applied Research Applications

CAMEO-4 is available for up to 19 individual lympho-hematopoietic cell populations derived from:

- Bone marrow
- Peripheral blood
- Umbilical cord blood
- Spleen
- Fetal liver
- Yolk sac

and obtained from:

- Non-human primate
- Horse
- Pig
- Sheep
- Dog
- Rat
- Mouse

In addition, purified cell populations from the aforementioned organs and tissues can also be used.

B. CAMEO™-4 for stem cell processing laboratories

Four CAMEO™-4 assay kits have been developed that are equivalent to corresponding MethoCult® reagents that are used in stem cell transplantation and cord blood bank processing laboratories.

TABLE 1
CAMEO™-4 Equivalent Assay Kits for Stem Cell processing Laboratories

CAMEO™-4 Catalog Number	Equivalent MethoCult® Reagent	Growth Factor/Cytokine Cocktail
KC-GEM2-50H	H4434	EPO, GM-CSF, IL-3, SCF
KC-GEM3-50H	H4034	EPO, GM-CSF, G-CSF, IL-3, SCF
KC-GM1-50H	H4534	GM-CSF, IL-3, SCF
KC-GM2-50H	H4035	GM-CSF, G-CSF, IL-3, SCF

These assay kits are only for human normal and mobilized peripheral blood, umbilical cord blood and bone marrow and purified cell populations (e.g. $CD34^+$ cells) from these tissues.

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

TABLE 3
Suggested Culture Incubation Times

Species	Cell Type	Cell Populations	Incubation Period (days)
Human	Bone marrow, normal and mobilized peripheral blood, umbilical cord blood	Stem and progenitor cells Precursor cells	10-14 7
Non-human primate	Bone marrow, peripheral blood	Stem and progenitor cells Precursor cells	10-14 7
Horse, Pig, Sheep, Dog	Bone marrow, peripheral blood	Stem and progenitor cells Precursor cells	10-14 4-7
Rat, Mouse	Bone marrow	Stem and progenitor cells Precursor cells	7 2-4

STEP 3. Colony Enumeration

After incubation, enumerate the colonies under an inverted microscope per your institution's standard operating procedure for the CFC assay.

STEP 2. CAMEO™-4 Cell Culture

Please refer to Section 8 for recommendations and tips prior to beginning this stage of the procedure.

The CAMEO™-4 Kit contains a Master Mix containing all of the reagents to culture cells under clonal conditions.

A. The CAMEO™-4 Method

1. Transfer the bottle of frozen CAMEO™-4 Master Mix to a 37ºC incubator or water bath to thaw at room temperature.
2. When thawed, mix the contents of bottle thoroughly, but try not to cause bubbles or foaming.
3. Prepare the cell suspension as required and adjust to the preferred working cell concentration.
4. Prepare and label tubes.
5. Prepare and label the culture plates.
6. When CAMEO™-4 Master Mix has thawed and has been mixed, dispense 0.54ml accurately into each tube.
   NOTE: *If using a CAMEO™-4 Assay Kit with no growth factors, first dispense only 0.48ml of the CAMEO™-4 Master Mix into the tube(s). If adding a specific growth factor/cytokine cocktail, ensure that the working and final concentrations are prepared so that 0.06ml can be added to produce a final volume of 0.54ml prior to adding the cell suspension. Use the IMDM supplied with the assay kit to ensure that the correct volumes are added.*
   IMPORTANT: *DO NOT use a syringe and needle to dispense the methylcellulose-containing Master Mix as this is extremely inaccurate and results in high coefficients of variation (%CV). HemoGenix® strongly recommends using a positive displacement repeater pipette for this purpose (see page 8).*
7. Now dispense 0.06ml of the working concentration cell suspension into the CAMEO™-4 Master Mix using a calibrated pipette. This now produces the Culture Master Mix.
   IMPORTANT: *If using manual pipettes, ensure that the mechanism is working correctly and that the pipette is properly calibrated. HemoGenix® strongly suggests using electronic pipettes .*
8. Mix the Culture Master Mix thoroughly by vortexing and leave for a few minutes for the mix to settle..
9. Using a positive displacement repeater pipette, dispense 0.1ml of the Culture Master Mix into each of the 4 replicate wells of each plate.
10. Using the same syringe tip, disperse the Culture Master Mix in the well so that the Mix completely covers the growth surface of the well
11. To prevent the culture plates from drying out, either transfer 2 plates to a sterile 100mm Petri dish containing an open 35 mm Petri dish filled with about 2-3ml of sterile water, or transfer all plates to a large container containing a beaker of water and cover the container with aluminum foil.
12. Incubate at 37˚C in a fully humidified incubator containing an atmosphere of 5% CO_2. If possible, use a 3-gas incubator to displace the atmospheric oxygen concentration (21%) to 5% O_2 with nitrogen. This helps to increase the plating efficiency by reducing oxygen toxicity to the cells. Table 3 shows the suggested incubation times.

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

3. The CAMEO™-4 PRINCIPLE

Whereas the "classic" CFC/CFU assay utilizes 35mm Petri dishes in which 1ml of reagents containing target cells are suspended in methylcellulose in each of 2-3 replicate dishes, CAMEO™-4 is a miniaturized form of this assay which only requires a total of 0.6ml to be prepared, from which 4 x 0.1ml replicate wells are prepared in a single, specialized 35mm Petri dish.

The specialized 35mm Petri dishes contain 4 wells with very low walls, which allow colonies to be easily viewed, differentiated and counted up to the edge of the well. The culture plates are included with the assay kit.

The cells are incubated for the same length of time as the "classic: CFC/CFU assay (usually 7-14 day for human cells and 2-7 days for animal cells depending on the cell population being detected).

4. OVERVIEW OF THE CAMEO™-4 PROCEDURE

There are 3 steps to using the CAMEO™-4 Kit.

Step 1 – Cell Preparation.
Cells are not provided with the CAMEO™-4 Kit. Prepare the cells according to a user-defined or pre-validated protocol. After ascertaining the cell count and viability, the cells are adjusted to a specific cell concentration

Step 2 – Cell Culture
The CAMEO™-4 Master Mix for the individual cell population being detected contains all the reagents required for the cell population to grow in culture. Alternatively, CAMEO™-4 is also available without growth factors and/or cytokines, so that the user can add their own.

The number of samples to be measured is determined and sterile, 5ml tubes labeled. A total of 0.54ml of the CAMEO™-4 Master Mix is dispensed into each tube and 0.06ml of cell suspension, adjusted to the correct concentration, added to the Master Mix. The contents of the tube are well mixed by vortexing. A total of 0.1ml is dispensed into each of the 4 replicate wells of the culture plates provided with the assay kit. This should be performed using a positive displacement repeater pipette. Syringes and needles should not be used. The cultures are incubated at 37°C in a fully humidified atmosphere containing 5%CO_2 and, if possible, 5% O_2. The use of low oxygen tension significantly improves plating efficiency by reducing the production of oxygen free radicals and therefore oxidative stress and DNA damage.

Step 3 – Once the incubation period has elapsed, the colonies are manually counted in all 4 replicate wells using an inverted microscope.

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

TABLE 1
Recommended Cell Doses for Different Species, Cell Types, Cell Preparations and Cell States for CAMEO™-4

Species	Cell Type	Cell Preparation	Cell State	Working Cell Concentration Required (100 x Final Cells/Well)	Final Cell Dose / Well
Human	Bone marrow	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
	Peripheral blood	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
	Mobilized peripheral blood	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
	Umbilical cord blood	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
	Umbilical cord blood	MNC	Frozen	0.5-0.75 x 10^6	5,000-7,500
	Bone marrow	$CD34^+$	Fresh	0.1-1 x 10^5	100-1,000
	Mobilized peripheral blood*	$CD34^+$	Fresh/ Frozen	0.1-5 x 10^5	100-5,000
	Umbilical cord blood	$CD34^+$	Fresh/ Frozen	0.1-5 x 10^5	100-5,000
Non-human primate	Bone marrow	MNC	Fresh/ frozen	0.5-0.75 x 10^6	5,000-7,500
	Peripheral blood	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
Horse, Pig, Sheep	Bone marrow	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
	Peripheral blood	MNC	Fresh/ frozen	0.5-0.75 x 10^6	5,000-7,500
Dog	Bone marrow	MNC	Fresh/ Frozen	0.5-0.75 x 10^6	5,000-7,500
Rat	Bone Marrow	MNC	Fresh	0.5-0.75 x 10^6	5,000-7,500
	Peripheral blood	MNC	Fresh	0.5-0.75 x 10^6	5,000-7,500
Mouse	Bone Marrow	MNC	Fresh	0.5-0.75 x 10^6	5,000-7,500
	Spleen	MNC	Fresh	0.5-1 x 10^6	5,000-10,000
	Fetal liver	MNC	Fresh	0.5-0.75 x 10^6	5,000-7,500

* Lot dependent

proximal end and immerse the whole bone in the medium contained in the tube.

6. Flush out the marrow through the bone cavity and withdraw part of the cell suspension through the bone and into the syringe.
7. Flush the cell suspension through the bone and repeat steps 6 and 7 two to three times. When finished, the bone should appear translucent, indicating that most of the cells have been flushed out of the cavity.
8. Remove the empty bone and replace it with the next bone until the marrow from all bones has been flushed out of the cavities.
9. Let the cell suspension settle for 1-2 minutes to allow large debris to fall to the bottom of the tube.
10. Using a small gauge (22-25) needle and syringe, slowly withdraw the cell suspension leaving the large debris in the tube and transfer it to a new tube, noting the volume.
11. If necessary, add medium to achieve the required volume.

To further purify a cell suspension, it is recommended to underlay 1-2ml of NycoPrep 1.077 (Axis-Shield) under the cell suspension in a sterile, 15ml conical tube with screw cap and centrifuge the cells according to the manufacturer's protocol.

C. Isolation of Hematopoietic Subpopulations

Providing sufficient cells are available, subpopulations of stem and progenitor cells can be isolated and purified for use in the HALO® assay. We recommend using magnetic cell isolation procedures (e.g. Miltenyi Biotech), since these allow rapid isolation of different cell populations with substantial purity, viability and yield. Please see table of recommended cell concentrations to determine the optimal, final cell concentration to use in the CAMEO™-4 procedure.

D. Cell Viability, Cell Counting and Cell Culture Suspension Preparation

1. For dye exclusion viability methods, use trypan blue and a hemocytometer or automated method, flow cytometry using 7-AAD or another vital stain.
   Note *that dye exclusion viability methods detect membrane integrity. They do not detect cellular and mitochondrial integrity and therefore metabolic viability.*
   A viability of 85% or greater should be obtained when using dye exclusion viability methods only. It is recommended not to use cell suspensions with a viability of less than 85% since these cells will not be able to sustain proliferation ability. Use ProliferSTEM™ (HemoGenix®, Inc) as a metabolic viability assay.
2. Determine the cell concentration using either a hemocytometer or electronic cell/particle counter.
3. Adjust the cell suspension concentration to that recommended in Table 1.
   Note *the working cell concentration per ml is 100 x the final cell concentration per well.* If cells have been treated prior to cell culture, higher cell concentrations may be required.
4. Prepare the total volume of cell suspension required using IMDM or PBS. The volume of the adjusted cell suspension required will be approx.10% of the total volume of CAMEO™-4 Culture Master Mix prepared.

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

5. ASSAY KIT CONTENTS and STORAGE CONDITIONS

Important

All assay kit components are quality controlled prior to use. HemoGenix does not take responsibility for the quality of reagents if used beyond the stated expiry date.

CAMEO™-4 Assay Kits contain:

- CAMEO™-4 Master Mix specific for cell population to be detected.
- IMDM Medium.
- 50 x 4-well, 35mm Petri dishes.
- Instruction manual.

Kits should be stored at -20°C until ready to use.

Once opened, the CAMEO™-4 Master Mix can be stored at 4°C. The CAMEO™-4 Master Mix should not be used after the expiry date has elapsed.

Extra culture plates can be purchased from HemoGenix®.

Good laboratory practices and universal protective precautions should be undertaken at all times when handling the kit components as well as cells and tissues. Material safety data sheets (MSDS) are included in each literature packet.

6. EQUIPMENT, SUPPLIES AND REAGENTS REQUIRED BUT NOT PROVIDED

Equipment and Supplies

1. Inverted microscope best fitted with 10x oculars and a 4x and 10x objectives
2. Laminar Flow Biohood.
3. Sterile plastic tubes (5ml, 10ml).
4. Single channel pipettes, preferably electronic (e.g. ViaFlow or Rainin EDP pipettes for variable volumes between 1µl and 1000µl).
5. Sterile pipette tips.
6. Repeater pipette with positive displacement (e.g. Oxford, Gilson Distriman or Rainin AutoRep E).
7. Sterile syringes for repeater pipette.
8. Vortex mixer.
9. Tissue culture incubator, humidified at 37°C with 5% CO_2 (minimum requirement) and 5% O_2 (preferable).
10. Hemocytometer or electronic cell counter to determine cell concentration.
11. Flow cytometer or hemocytometer for determining viability.

Reagents

1. Sterile Phosphate Buffered Saline (PBS)
2. Iscove's Modified Dulbecco's Medium (IMDM)
3. Density-gradient medium (e.g. NycoPrep 1.077, Axis-Shield).
4. 7-AAD, propidium iodide or trypan blue for viability assay.

HemoGenix® Changing the Paradigm
Assays You Can Trust
Innovative Expertise You Can Count On

7. The CAMEO™-4 PROTOCOL

PLEASE READ THE FOLLOWING PROTOCOL CAREFULLY
SEE SECTION 8 BEFORE PERFORMING THE PROTOCOL

Performing CAMEO™-4 is a 3-step process.

Step 1 – Cell preparation.
Step 2 – Addition of cells to the CAMEO™-4 Master Mix
Step 3 – Enumeration of colonies.

Step 1 and Step 2 must be performed in a laminar flow biohazard hood

STEP 1 – Cell Preparation

A. Human, Non-Human Primate, Horse, Pig, Sheep or Canine Cells

1. For best results, CAMEO™-4 requires that target cells be separated from red blood cells. Red blood cells should be removed because these can interfere with colony growth as well as make it extremely difficult to count colonies. Neutrophils and platelets should also be removed. A mononuclear cell (MNC) suspension is the cell suspension of choice. HemoGenix® recommends separating the MNCs using NycoPrep 1.077 (Axis-Shield) density gradient centrifugation media. This separation procedure should be used for human, non-human primate, horse, pig, sheep and canine cells. Ficoll-Paque can also be used, although this is toxic to cells. Follow the manufacturer's protocol to prepare the MNCs. A hematocrit of 10% or less is required to avoid interference by red blood cells.
2. Resuspend the cells in IMDM or PBS

Human umbilical cord blood, peripheral blood(normal/mobilized) or bone marrow cells.
It is recommended to deplete the cord blood of erythrocytes using a current Hetastarch® protocol or a density gradient separation per the manufacture's protocol so that the majority of the erythrocytes are removed from the cell suspension. It is not recommended to perform the CFC assay with a high concentration of erythrocytes present.

B. Rat or Murine Bone Marrow

1. Remove organs (femora and tibia (optional)) under aseptic conditions.
2. Remove as much muscle from the bones as possible.
3. Using a sterile blade, first cut off the proximal (hip joint) end below the ball joint at right angles to the longitudinal length of the bone. Then cut off the distal end (above the patella or knee).
4. Transfer sufficient sterile medium to a tube so that it will cover the whole bone, approximately 1-2ml. (Some of the medium provided with the kit can be used for this purpose).
5. Half fill a syringe (1-3ml) with sterile medium and, using a needle gauge that will enter the bone cavity without cracking the bone, insert the needle into the