AllColonies™ Traditional

Hematopoietic Multi-Stem, Multi-Lineage Methylcellulose Colony-Forming Cell (CFC) Assay for 35mm Petri Dish Format

Technical Manual

(Version 8-19)

This manual should be read in its entirety prior to using this product

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

Preferred Cell Systems™

KM009.001

TABLE OF CONTENTS

1. LIMITATIONS OF THE ASSAY AND PRECAUTIONS

1. *AllColonies™ is not approved by either the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA)*
2. *AllColonies™ is for research use only and have not been approved for clinical diagnostic use.*
3. *Reagents are STERILE. Perform all procedures under sterile conditions, except where indicated.*
4. *These reagents should not be used beyond the expiration date on the label.*
5. *Always use professionally calibrated and, preferably, electronic pipettes for all dispensing procedures. Small discrepancies in pipetting can lead to large pipetting errors. Although electronic pipettes self-calibrate themselves, they still need to be professionally calibrated on a regular basis.*
6. *Good laboratory practices and universal protective precautions should be undertaken at all times when handling the kit components as well as human cells and tissues. Material safety data sheets (MSDS) are included in each literature packet.*

2. Introduction

AllColonies™ is a methylcellulose reagent for the clonal growth of multiple stem, progenitor and precursor cells from the lympho-hematopoietic system. These are termed colony-forming cell (CFC) or colony-forming unit (CFU) assays.

AllColonies™ is a ready-to-use reagent that contains a proprietary cocktail of growth factors, cytokines and other supplements that stimulate the production of multiple colony types, from stem cells to precursor cells, in a single culture. AllColonies™ is available in the traditional 35mm Petri dish format, a miniaturized (Micro) format and a 96-well plate (Multi) format.

AllColonies™ can be used for stem cell, basic and veterinary research applications and hematopoietic cellular therapy applications in the stem cell processing laboratory. However, AllColonies™ is a more flexible and general CFC/CFU reagent for cellular therapy applications allowing colonies derived from multiple primitive cell populations to be detected and enumerated.

The principle of the CFC/CFU assay is to suspend hematopoietic cells in methylcellulose containing growth factors and/or cytokines . During culture, the cells proliferate, but are immobilized in viscous methylcellulose. As a result, aggregates of dividing cells form into colonies. The size of the colony is an indication of the primitiveness of the cell producing the colony. After a specific incubation time, the colonies are numerated using an inverted microscope. The number of colonies produced correlates with the number of cells plated and the concentration of the growth factors/cytokines used to stimulate the cells.

3. Use of AllColonies™

AllColonies™ can be used for virtually any application requiring the clonal culture of hematopoietic cells where multiple colony types need to be enumerated in a single culture dish. AllColonies™ is particularly useful in the cell processing laboratory, where stem, progenitor and precursor cell colonies can be enumerated simultaneously.

AllColonies™ can be used with cells from the following tissue sources:

- Embryonic tissue
- Fetal tissue
- Spleen
- Bone marrow
- Peripheral blood
- Cord blood

AllColonies™ is available for cells derived from the following species:

- Human
- Non-human primate
- Dog
- Rat
- Mouse

4. Colony Types and Cells Detected Using AllColonies™

Primitive hematopoietic stem and progenitor cells are rare. They cannot be identified in a microscope. Their presence is determined by their functional ability to produce colonies of differentiated and mature cells that identify the parent cell that gave rise to the colony. AllColonies™ contains a proprietary cocktail of growth factors, cytokines and supplements that allow a large array of different primitive cell types to be stimulated. The types of colonies produced are dependent upon a number of factors. These include, but are not limited to:

- Fractionation or purification of the tissue being used.
- The quality of cells after fractionation and/or purification.
- Metabolic and functional status of the tissue at the time of use.
- Tissue source
- Species

The types of colonies produced might include, but are not limited to:

- Multipotential stem cell colonies
- Bi- and tri-potential stem cell colonies
- Granulocyte colonies (G-CFC)
- Macrophage colonies (M-CFC)
- Bi-lineage colonies of granulocytes and macrophages (GM-CFC)
- Mature erythroid colonies (CFU-E)
- Primitive erythroid colonies (BFU-E)
- Megakaryocyte colonies (Mk-CFC)
- T-lymphocyte colonies (T-CFC)
- B-lymphocyte colonies (B-CFC)

5. Overview of the AllColonies™ Traditional Procedure

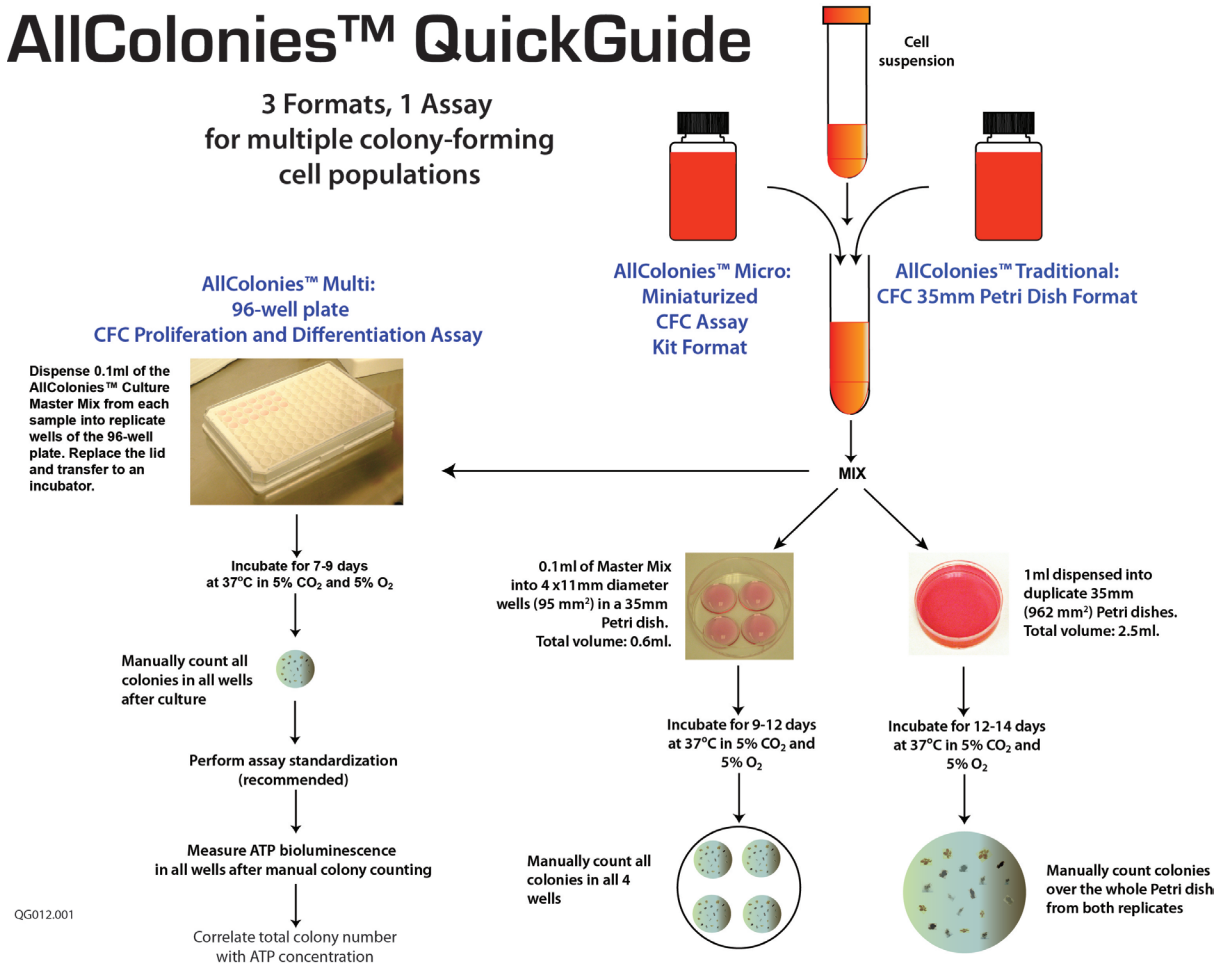
Using AllColonies™ Traditional is a 3 step process.

Step 1 – Cell Preparation
Cells are not provided with AllColonies™. Cells are prepared either by using a user-defined, pre-validated protocol to obtain a single cell suspension or procedures that are suggested in this manual. A dye exclusion viability and/or metabolic viability and nucleated cell count should be performed on all samples.

Step 2 – Cell Culture
AllColonies™ is a complete, ready-to-use methylcellulose reagent. The reagent is usually provided in a bottle containing 100mL. For each sample to be tested, a total of 2.25mL of AllColonies™ reagent is dispensed into a sterile 5mL plastic tube followed by 0.25mL (10% of the total culture volume) of the cell suspension adjusted to the correct cell concentration. The contents of the tubes are mixed thoroughly on a vortex mixer and 1mL is dispensed into each of two 35mm Petri dishes. The plates are transferred to a 37°C, humidified incubator gassed with 5% CO_2 and preferably 5% O_2. Using low oxygen tension to grow hematopoietic cells reduced oxygen toxicity due to free radical formation, thereby improving the plating efficiency.

Step 3 - Colony Counting
For animal cells, cultures are incubated for about 5-7 days. For human cells, cultures are incubated for 9-12 days. After incubation, colonies are enumerated, by counting either the total number or differentiating the colonies into different types to produce a total colony count.

6. QuickGuide to AllColonies™



AllColonies™ is available in 3 formats:

- Traditional 35mm Petri dish format.
- Miniaturized (Micro) format.
- 96-well plate (Multi) format.

The present technical manual describes the Traditional format.

7. AllColonies™ Reagent and Storage

The AllColonies™ Traditional volume/bottle is 100mL. AllColonies™ is shipped frozen. Upon arrival, transfer the bottle to a -20°C freezer until used. At -20°C, the reagent can be kept for 1 year or until the expiry date. Once thawed, AllColonies™ can be kept at 4°C for 1 month. Do not repeatedly refreeze and thaw the AllColonies™ reagent.

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.

8. Equipment, Supplies and Reagents Required, but not Provided

Equipment and Supplies

1. 35mm Petri dishes that are not tissue culture treated.

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

Reagents

1. Sterile Phosphate Buffered Saline (PBS)
2. HemoGro™ low serum medium (Preferred Cell Systems™) or Iscove's Modified Dulbecco's Medium (IMDM)
3. Density-gradient medium (e.g. LymphoPrep).
4. 7-AAD, propidium iodide or trypan blue or other dye exclusion viability assay.
5. LIVEGlo™ metabolic viability assays (Preferred Cell Systems™)

9. The AllColonies™ Traditional Protocol

PLEASE READ THE FOLLOWING PROTOCOL CAREFULLY.
SEE SECTION 10 BEFORE PERFORMING THE ASSAY

Good laboratory practices and universal protective precautions should be undertaken at all times

Performing a ColonyGro™ Traditional CFC/CFU assay is a 3 step process.

Step 1 – Cell preparation.
Step 2 – Culture preparation, plating and incubation in 35mm Petri dishes.
Step 3 - Colony counting.

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

STEP 1 – Cell Preparation

A. Human, Non-Human Primate, Dog, Rat and Mouse Cells

1. For best results using bone marrow or normal peripheral blood, AllColonies™ 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. Therefore, fractionation of the cell suspension to a mononuclear cell (MNC) suspension is highly recommended. Fractionation should be performed for human, primate, dog and rat. Mouse bone marrow cells contain few red blood cells and do not usually require a MNC fraction to be tested in the AllColonies™ assay.
2. Resuspend the cells in HemoGro™ or IMDM prior to cell counting and viability testing.

B. Human Umbilical Cord Blood, Bone Marrow and Mobilized Peripheral Blood Used for Cellular Therapy.
Human umbilical cord blood is usually red blood cell and/or plasma reduced. This produces a so-called total nucleated cell (TNC) fraction that contains platelets, granulocytes and sometimes more than 30% red blood cells. This fraction can be used for the AllColonies™ assay. However, it should be emphasized that studies demonstrate that the TNC fraction can dilute and mask the presence of primitive stem and progenitor cells, thereby severely underestimating the actual quality and functionality of the cell test preparation. It is therefore recommended that for testing purposes, a TNC sample should be

further processed to a MNC fraction.

The same considerations apply to testing human bone marrow for cellular therapy purposes.

After apheresis, mobilized peripheral blood usually has a similar fractionation status to MNCs and do not need further processing.

C. Cryopreserved Cells

Cryopreserved cells can be used in the AllColonies™ assay.

If cells are cryopreserved as a total nucleated cell product, they will contain red blood cells, granulocytes and other cell populations. When the cells are thawed, granulocytes and other cell components will rupture and may release DNA. Large amounts of released DNA will clump together encasing cells. For cryopreserved MNC fractions or highly purified cells, the chances of clumping are usually low.

To reduce or alleviate the possibility of clumping during cell thawing, it is recommended that DNase be added to the cell suspension. The following procedure is used for small aliquots of cells only (1-1.5mL).

1. Thawing of the vial contents is initiated in a 37°C water bath, by swirling the vial for approx. 1 min.
2. When a small ball of ice still remains in the vial (1-2 min), remove the vial from the water bath, sterilized the outside of the vial by spraying with 70% ethanol and carefully unscrew the vial lid.
3. It is possible that clumping can occur at this stage, in which case, add DNase to the total volume in the vial to achieve a concentration of 6µg/mL before proceeding to the next step.
4. Using a 1mL pipette, gently mix the contents of the vial and transfer to a 50mL tube containing 20mL of thaw medium or HemoGro™ low serum medium. Up to 3 vials of the same cells can be added to this 20mL of thaw medium. However, clumping can also occur at this stage. In this case, DNase at a final concentration of 6µg/mL should be added before proceeding to the next step.
5. Gently mix the cells by swirling the contents of the tube. Do not use repeat pipetting to mix the cells. This could cause further rupture of cells and the release of DNA resulting in increased clumping.
6. Centrifuge the cells at 300 x g for 10 min at room temperature and discard the supernatant after centrifugation.
7. Resuspend the cells in 1mL of HemoGro™ or IMDM. If necessary, add 6µg/mL DNase.

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.
   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 the proliferation ability required for colony growth.
   Note *that dye exclusion viability methods detect membrane integrity. They do not detect cellular and mitochondrial integrity and therefore metabolic viability. Studies have shown that dye exclusion viability values even greater than 85% do not guarantee colony formation, growth, quality or potency of the cells. Only metabolic viability assays such as LIVEGlo™, HALO®, HemoFLUOR™ or HemoLIGHT™ can provide this information.*
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 10 x the final cell concentration per 35mm Petri dish.* 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 HemoGro™ or IMDM. The volume of the adjusted cell suspension required will be 10% of the total volume of the AllColonies™ reagent.

STEP 2. AllColonies™ Traditional Cell Culture

Please refer to Section 7 for recommendations and tips prior to beginning this stage of the procedure.

Perform all cell culture procedures under sterile conditions in a biosafety cabinet.

1. Transfer the bottle of frozen AllColonies™ reagent to a 37ºC water bath or allow to thaw at room temperature.
2. When thawed, mix the contents of bottle thoroughly by inversion several times.
3. Prepare the cell suspension as required and adjust to the preferred working cell concentration. Table 1 shows the working cell concentrations. Ideally, the final cell concentration should produce sufficient colonies to allow statistical significance between samples. This requires that a minimum of between 20-25 colonies should be obtained. However, this can vary depending on the cell source and species.
4. Prepare and label individual tubes for each sample to be tested.
5. Prepare and label duplicate, 35mm Petri dishes for each sample to be tested.
6. Using a positive displacement repeater syringe pipette, dispense 2.25 mL of the ready-to-use AllColonies™ reagent accurately into each tube. See Section 7 for important information on dispensing methylcellulose reagents.
   DO NOT *use a syringe and needle to dispense methylcellulose reagents. This is extremely inaccurate and results in high coefficients of variation (%CV). It is strongly recommended to use a calibrated positive displacement repeater syringe pipette for this purpose (See Section 7).*
7. Dispense 0.25 mL of the working cell concentration into each tube.
   IMPORTANT*: If using manual pipettes, ensure that the mechanism is working correctly and that the pipette is properly calibrated. It is suggested to use electronic pipettes if available, since these are self-calibrating.*
8. Mix the contents thoroughly by vortexing and leave to stand for a few minutes.
9. Using a positive displacement repeater syringe pipette (see Section 7), withdraw approximately 2.25-2.3 mL, taking care not to withdraw bubbles.
10. Accurately dispense 1.0 mL into the middle of each of the two 35mm Petri dishes.
11. Leave to spread over the surface of the Petri dish. To help spread the contents evenly over the surface of th dish, gently rock the Petri dish so that culture reagent covers the surface completely.
12. To prevent the Petri dishes from drying out, transfer both dishes 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.
13. Incubate the cells 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 increases the plating efficiency by reducing oxygen toxicity to the cells. Table 2 shows the suggested incubation times.

TABLE 1
Recommended Cell Concentrations

Species	Cell Type	Cell Preparation	Cell State	Working Cell Concentration Required (10 x Final Cells/Well)	Final Cell Concentration / Well
Human	Bone marrow	MNC	Fresh/ Frozen	0.1-2 x 10^6	0.1-2 x 10^5
	Peripheral blood	MNC	Fresh/ Frozen	0.5-2 x 10^6	0.5-2 x 10^5
	Mobilized peripheral blood	MNC	Fresh/ Frozen	0.1-2 x 10^6	0.1-2 x 10^5
	Umbilical cord blood	MNC	Fresh/ Frozen	0.1-2 x 10^6	0.1-2 x 10^5
	Bone marrow	$CD34^+$	Fresh	0.1-1 x 10^5	0.1-1 x 10^4
	Mobilized peripheral blood*	$CD34^+$	Fresh/ Frozen	0.1-1 x 10^5	0.1-1 x 10^4

Species	Cell Type	Cell Preparation	Cell State	Working Cell Concentration Required (10 x Final Cells/Well)	Final Cell Concentration / Well
	Umbilical cord blood	$CD34^+$	Fresh/ Frozen	0.1-1 x 10^5	0.1-1 x 10^4
Non-human primate	Bone marrow	MNC	Fresh/ frozen	0.1-2 x 10^6	0.1-2 x 10^5
	Peripheral blood	MNC	Fresh/ Frozen	1-5 x 10^6	1-5 x 10^5
Dog	Bone marrow	MNC	Fresh/ Frozen	0.5-2 x 10^6	0.5-2 x 10^5
Rat	Bone Marrow	MNC	Fresh	0.1-2 x 10^6	0.1-2 x 10^5
	Peripheral blood	MNC	Fresh	1-5 x 10^6	1-5 x 10^5
Mouse	Bone Marrow	MNC	Fresh	0.1-2 x 10^6	0.1-2 x 10^5
	Spleen	MNC	Fresh	1-10 x 10^6	1-10 x 10^5
	Fetal liver	MNC	Fresh	0.1-2 x 10^6	0.1-2 x 10^5

TABLE 2
Suggested Culture Incubation Times

Species	Cell Type	Cell Populations	Incubation Period (days)
Human	Bone marrow, normal and mobilized peripheral blood, umbilical cord blood	Stem cells Progenitor cells Precursor cells	9 - 12 9 - 12 5 - 7
Non-human primate	Bone marrow, peripheral blood	Stem cells Progenitor cells Precursor cells	9 - 12 9 - 12 5 - 7
Dog, Rat, Mouse	Bone marrow	Stem cells Progenitor cells Precursor cells	5 - 7 5 - 7 2-4

STEP 3 - COUNTING COLONIES

Table 2 shows the incubation times for different hematopoietic tissues derived from different species. It is important to emphasize that the number of colonies is determined by the number of colony-forming cells present in the cell suspension. Therefore, the number of colonies that can be produced is "set in stone" very early in the culture. Increasing the time period does not increase the number of colonies; it only increases the size of the colonies and the state of differentiation and maturation of the cells that identify the colonies.

A colony is usually defined as an aggregate of 8 or more cells. This represents a minimum of 3 sequential divisions performed by the original colony-forming cell. There are three ways of counting colonies grown in methylcellulose.

1. ***Count each individual center within a colony***. Colonies are rarely spherical with a single dark center indicating the center of proliferation. Most colonies are irregular in shape and contain many dark centers. Each dark center represents a single "proliferation unit" (PU) in which the cells are actually proliferating. With time, these PUs grow together to form an irregular-shaped colony. Counting the individual PUs within a colony provides an indication of colony proliferation. However, this should not be misunderstood as a means to quantify cell proliferation. Although colony formation requires cell proliferation, the colony-forming assays is not a cell proliferation assay and does not measure cell proliferation.
2. ***Count all PUs within a colony as 1 colony***. This is the normal manner in which colonies are counted. No consideration is made regarding whether the colony is spherical or irregular in shape. If it is separate entity from another colony, it is counted as a single colony.
3. ***Use a camera fitted to a microscope or an electronic colony counter*** to image the colonies and save the image for later

enumeration either to count the colonies manually or using image analysis software. This type of colony enumeration can be problematic since the walls of the well usually cast shadows around the outer rim of the well. In addition, the software may have to be "taught" how to count colonies. If this type of colony enumeration is to be performed, it is suggested to compare manual counting with electronic counting to ensure that the correct number of colonies are counted in both instances.

Important Considerations When Evaluating Colony-Forming Cell Results

1. The number of colonies counted provides no quantitative information on cell proliferation. Cell proliferation is inferred, since without cell proliferation, no colonies would be obtained.
2. The CFC/CFU assay detects cell differentiation since the colonies can only be identified by the ability of the cells to differentiate and mature.
3. Monitor the growth of colonies. Colonies should be counted at a time when they can be individually identified. Longer incubation times only increases the size of the colonies, which then grow together making it very difficult to identify individual colonies.
4. In general, the size of the colony is indicative of the primitiveness of the cell that produced the colony; that is, the larger the colony, the more primitive the cell.
5. Colony evaluation requires considerable time to learn. If performing a colony assay for the first time, ask a colleague of contact Preferred Cell Systems™ for advice.

10. Recommendations and Tips Prior to Using AllColonies™.

(i) Cell Suspension

- The preferred cell suspension is a mononuclear cell suspension (MNC). Murine peripheral blood should also be a MNC preparation. However, murine bone marrow can usually be used without any fractionation.
- Human cells. Many cell therapy applications use a total nucleated cell (TNC) fraction that can contain more than 30% red blood cells and high numbers of granulocytes and platelets. This TNC fraction can be used in the AllColonies™ assay. However, using the TNC fraction can severely underestimate the number and types of colonies capable of being formed. It is highly recommended that for ease of colony counting and more reliable results, the cells be further purified to a MNC fraction.
- High concentrations of red blood cells can make it extremely difficult to view and identify colonies and can result in false negatives.
- If cells have been treated (e.g. with cytotoxic drugs etc.) prior to cell culture, higher cell concentrations than those shown in Table 1 may be required.

(ii) Thawing and Storage of the AllColonies™

- Prior to using the AllColonies™, remove the bottle from the freezer and thaw either at 37°C or at room temperature.
- After thawing, AllColonies™, is stable at 2-8°C for 1 month after thawing.

(iii) Dispensing AllColonies™ Reagent

Methylcellulose is notoriously difficult to dispense accurately. DO NOT use a syringe and needle to dispense any methylcellulose reagent, since it will result is serious dispensing errors and high coefficients of variation (CVs). It is recommended to use positive displacement (preferably electronic) repeater, syringe pipettes to dispense all methylcellulose reagents.

(iv) Number of Replicates Performed

AllColonies™ has been designed for samples to be tested in duplicate (2 replicates). The total volume, including cell suspension, for each sample prepared is 2.5mL. Sufficient AllColonies™ Reagent is provided for up to 44 samples.

(v) Humidity Chamber

Duplicate cultures should be placed in a 100mm sterile Petri dish humidity chamber containing a 35mm Petri dish without a lid and filled with 2-3mL of sterile water. Even fully humidified incubators do not provide sufficient humidity to prevent evaporation of the culture reagents over long periods of time.

(vi) Incubation Times

The culture time depends on a number of different factors, including cell source, species and cell concentration. The plates can be removed at any time from the incubator to monitor colony growth. Do not allow the colonies to grow into each other. It will be difficult to count the colonies. The cells should be cultured until the colony type can be identified and the colonies have grown so that they can be discreetly counted individually.

11. Troubleshooting

1. Colonies grow together so that they cannot be counted

The source of the cells used, the cell density and the incubation time will define the growth of the cells and colony formation. To count and differentiate colonies with any degree of accuracy, it is important that each colony is distinct and separated from its neighbor. If colonies grow into each other so that they cannot be properly counted, the cultures have been left in the incubator too long. Either reduce the incubation time or the cell density so that the number of colonies can be counted correctly.

2. Colony growth is rapid

Unlike other methylcellulose formulations, AllColonies™ has been designed for rapid colony growth. It will be noticed that the methylcellulose is more fluid than that of other formulations. In other words, the methylcellulose is less viscous. The more viscous the methylcellulose, the more difficult it is for the cells comprising the colony to expand. This can lead to reduced colony size or prevent colony formation all together. Monitor colony growth until and optimum incubation time is reached. However, the first 24 - 48 hours days are the most critical for colony growth. For hematopoietic stem and progenitor cells, do not remove the cultures from the incubator for at least 3 - 4 days.

3. Cultures dry out

An incubator may be considered "fully humidified" . However, the humidity throughout an incubator may not be equal. To ensure humidity, place the culture plates 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.

4. High replicate or experimental variation

- This is usually caused by several factors. The first is variations due to dispensing methylcellulose. Methylcellulose is notoriously difficult to dispense accurately. Do not use syringes and needles. This method is inaccurate and can lead to extremely high variations. Use a positive displacement repeater syringe pipette to dispense all methylcellulose reagents. Also ensure that all pipettes are properly calibrated. Small errors in dispensing can lead to large variations.
- Ensure that cell concentration calculations have been performed properly and that 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.

Ordering Information
Toll free: 1-888-436-6869
Tel: (719) 264-6251
Fax: (719) 264-6253
Email: orders@preferred-cell-systems.com
Order online at preferred-cell-systems.com

Technical Support
Tel: (719) 264-6251
Email: info@preferred-cell-systems.com

Preferred Cell Systems™
1485 Garden of the Gods Road
Suite 152
Colorado Springs, CO 80907
U.S.A.
Website: www.preferred-cell-systems.com

Preferred Cell Systems™