

**Assays-in-a-Box™**  
by  
**Preferred Cell Systems™**

# ColonyGro™

Hematopoietic Methylcellulose  
Colony-Forming Cell (CFC) Assays

## Technical Manual

(Version 9-23)

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

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

No part of this instruction manual may be copied, duplicated or used  
without the express consent of Preferred Cell Systems™

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## 1. LIMITATIONS OF THE ASSAY AND PRECAUTIONS

1. *ColonyGro™ is not approved by either the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA).*
2. *ColonyGro™ is for research use only and has 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

ColonyGro™ 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.

ColonyGro™ has ready-to-use reagents available with different cocktails of growth factors and cytokines that stimulate the production of colonies derived from stem cell, progenitor, or precursor cells. ColonyGro™ is a 35mm Petri dish assay.

ColonyGro™ can be used for stem cell, basic, and veterinary research applications and hematopoietic cellular therapy applications in the stem cell processing laboratory.

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 enumerated 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 ColonyGro™

ColonyGro™ can be used for virtually any application requiring the clonal culture of hematopoietic cells where multiple or specific colony types need to be enumerated. For the cellular therapy processing laboratory, ColonyGro™ is available with formulations similar to those of MethoCult™ where stem, progenitor, and precursor cell colonies can be enumerated simultaneously.

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

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

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

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

#### 4. Colony Types and Cells Detected Using ColonyGro™

Individual ColonyGro™ reagents are available to detect the following cell populations:

Cell Type	Cell Population	Growth Factors/Cytokines
	Not specified	No growth factors or cytokines included
Stem Cells	HPP-SP 1	IL-3, IL-6, SCF, TPO, Flt3-L
	HPP-SP 2	EPO, GM-CSF, IL-2, IL-3, IL-6, IL-7, SCF, TPO, Flt3-L
	CFC-GEMM 1	EPO, GM-CSF, IL-3, IL-6, SCF, TPO, Flt3-L
	CFC-GEMM 2	EPO, GM-CSF, IL-3, IL-6, SCF, TPO
	CFC-GEMM 3	EPO, GM-CSF, G-CSF, IL-3, IL-6, SCF + TPO
	CFC-GEM 1	EPO, GM-CSF, IL-3, IL-6, SCF
	CFC-GEM 2	EPO, GM-CSF, IL-3, SCF
	CFC-GEM 3	EPO, GM-CSF, G-CSF, IL-3, SCF
Progenitor Cells	BFU-E 1	EPO, IL-3, SCF
	BFU-E 2	EPO
	GM-CFC 1	GM-CSF, IL-3, SCF
	GM-CFC 2	GM-CSF, G-CSF, IL-3, SCF
	GM-CFC 3	GM-CSF
	Mk-CFC 1	TPO, IL-3, SCF
	B-CFC	IL-7
Precursor Cells	CFU-E	EPO
	G-CFC	G-CSF
	M-CFC	M-CSF
	Mk-CFC	TPO

Colony-Forming Unit - Granulocyte, Erythroid, Macrophage. BFU-E: Burst-Forming Unit - Erythroid. GM-CFC: Granulocyte-Macrophage Colony-Forming Cell. Mk-CFC: Megakaryocyte - Colony-Forming Cell. CFU-E: Colony-Forming Unit Erythroid. G-CFC: Granulocyte Colony-Forming Cell. M-CFC: Macrophage Colony-Forming Cell. B-CFC: B-lymphocyte Colony-Forming Cell. EPO: Erythropoietin. SCF: Stem Cell Factor. TPO: Thrombopoietin. CSF: Colony Stimulating Factor. IL: Interleukin.

#### *ColonyGro™ for stem cell processing laboratories*

Five ColonyGro™ reagents and CAMEO™-4 assay kits are available for stem cell processing laboratories that are equivalent to corresponding MethoCult® reagents.

**ColonyGro™ Equivalent Assay Kits for Stem Cell Processing Laboratories**

ColonyGro™ Catalog Number	Equivalent MethoCult® Reagent	Growth Factor/Cytokine Cocktail
CFC-GEMM3-100H	H4435	EPO, GM-CSF, G-CSF, IL-3, IL-6, SCF, TPO*
CFC-GEM2-100H	H4434	EPO, GM-CSF, IL-3, SCF
CFC-GEM3-100H	H4034	EPO, GM-CSF, G-CSF, IL-3, SCF
CFC-GM1-100H	H4534	GM-CSF, IL-3, SCF
CFC-GEM2-100H	H4035	GM-CSF, G-CSF, IL-3, SCF

\* Please note that thrombopoietin (TPO) is not included in H4435 formulation.

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

## 5. Overview of the ColonyGro™ Procedure

Using ColonyGro™ is a 3 step process.

### Step 1 – Cell Preparation

Cells are not provided with ColonyGro™. Cells are prepared either by using a user-defined, pre-validated protocol to obtain a single cell suspension or by 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

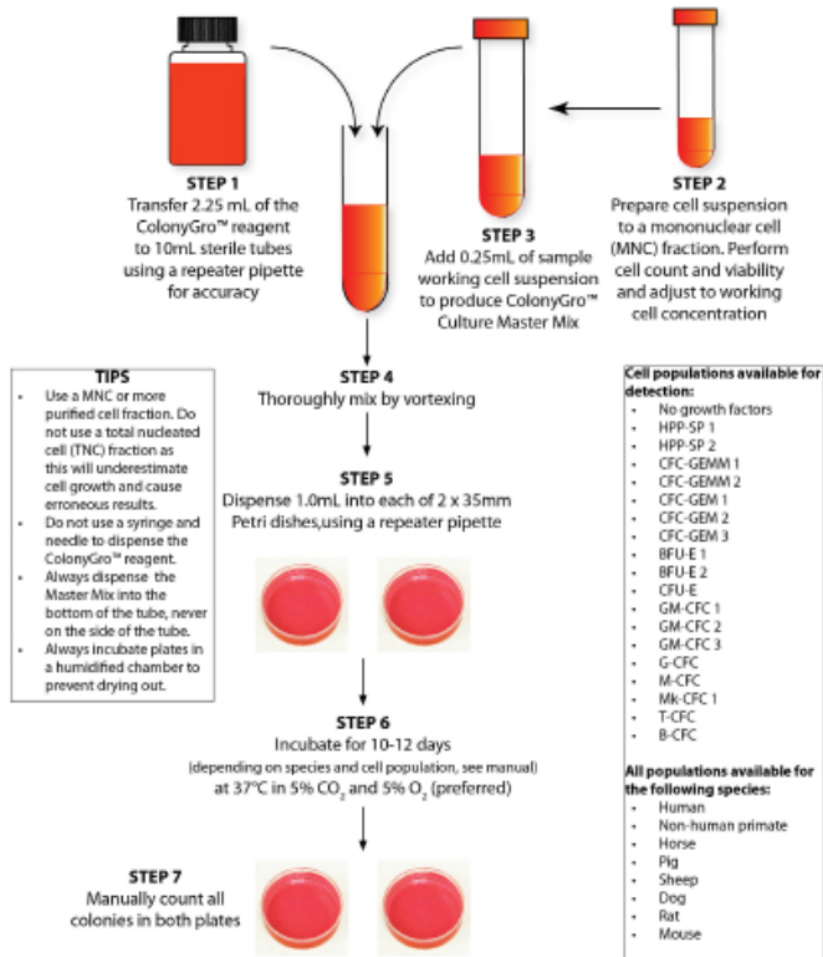
ColonyGro™ is a complete, ready-to-use methylcellulose reagent. The reagent is provided in a bottle containing 100mL. For each sample to be tested, a total of 2.25mL of ColonyGro™ reagent is dispensed into a sterile 5mL plastic tube followed by 0.25mL (10% of the total culture volume) of the cell suspension 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<sub>2</sub> and, preferably, 5% O<sub>2</sub>. 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 ColonyGro™

# QuickGuide to ColonyGro™



QG013.001

## 7. ColonyGro™ Reagent and Storage

The ColonyGro™ volume/bottle is 100mL. ColonyGro™ 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 (whichever comes first). Once thawed, ColonyGro™ can be kept at 4°C for 1 month. Do not repeatedly refreeze and thaw the ColonyGro™ 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 Streak, Oxford, Gilson Distriman or Rainin AutoRep E) for all procedures involving the dispensing of the ColonyGro™ methylcellulose reagent.
8. Sterile syringes (1mL) for repeater pipette.
9. Vortex mixer.
10. Tissue culture incubator, humidified at 37°C with 5% CO<sub>2</sub> (minimum requirement) and 5% O<sub>2</sub> (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. NycoPrep 1.077, Axis-Shield or Ficoll).
4. 7-AAD, propidium iodide or trypan blue, or other dye exclusion viability assay.
5. LIVEGlo™ metabolic viability assays (Preferred Cell Systems™).

## 9. The ColonyGro™ Protocol

**PLEASE READ THE FOLLOWING PROTOCOL CAREFULLY.  
SEE SECTION 7 BEFORE PERFORMING THE ASSAY.**

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



Performing a ColonyGro™ 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, Horse, Pig, Sheep, Dog, Rat and Mouse Cells**

1. For best results using bone marrow or normal peripheral blood, ColonyGro™ 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, horse, pig, sheep, 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 ColonyGro™ 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.**

1. 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 ColonyGro™ 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 does not need further processing.

**C. Cryopreserved Cells**

Cryopreserved cells can be used in the ColonyGro™ 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, sterilize 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.

- 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.
- 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.
- Centrifuge the cells at 300 x g for 10 min at room temperature and discard the supernatant after centrifugation.
- Resuspend the cells in 1mL of HemoGro™ or IMDM. If necessary, add an additional 6µg/mL DNase.

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

- 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: 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.*

- Determine the cell concentration using either a hemocytometer or electronic cell/particle counter.
- 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.*
- 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 ColonyGro™ reagent.

## STEP 2. ColonyGro™ 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.**

- Transfer the bottle of frozen ColonyGro™ reagent to a 37°C water bath or allow to thaw at room temperature.
- When thawed, mix the contents of bottle thoroughly by inversion several times.
- 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.
- Prepare and label individual tubes for each sample to be tested.
- Prepare and label duplicate, 35mm Petri dishes for each sample to be tested.
- Using a positive displacement repeater syringe pipette, dispense 2.25 mL of the ready-to-use ColonyGro™ 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 the dish, gently rock the Petri dish so that the 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<sub>2</sub>. If possible, use a 3-gas incubator to displace the atmospheric oxygen concentration (21%) to 5% O<sub>2</sub> 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 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>
	Peripheral blood	MNC	Fresh/Frozen	0.5-2 x 10 <sup>6</sup>	0.5-2 x 10 <sup>5</sup>
	Mobilized peripheral blood	MNC	Fresh/Frozen	0.1-2 x 10 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>
	Umbilical cord blood	MNC	Fresh/Frozen	0.1-2 x 10 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>
	Bone marrow	CD34 <sup>+</sup>	Fresh	0.1-1 x 10 <sup>5</sup>	0.1-1 x 10 <sup>4</sup>
	Mobilized peripheral blood*	CD34 <sup>+</sup>	Fresh/Frozen	0.1-1 x 10 <sup>5</sup>	0.1-1 x 10 <sup>4</sup>
	Umbilical cord blood	CD34 <sup>+</sup>	Fresh/Frozen	0.1-1 x 10 <sup>5</sup>	0.1-1 x 10 <sup>4</sup>
Non-human primate, horse, pig, sheep	Bone marrow	MNC	Fresh/frozen	0.1-2 x 10 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>
	Peripheral blood	MNC	Fresh/Frozen	1-5 x 10 <sup>6</sup>	1-5 x 10 <sup>5</sup>

Species	Cell Type	Cell Preparation	Cell State	Working Cell Concentration Required (10 x Final Cells/Well)	Final Cell Concentration / Well
Dog	Bone marrow	MNC	Fresh/Frozen	0.5-2 x 10 <sup>6</sup>	0.5-2 x 10 <sup>5</sup>
Rat	Bone Marrow	MNC	Fresh	0.1-2 x 10 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>
	Peripheral blood	MNC	Fresh	1-5 x 10 <sup>6</sup>	1-5 x 10 <sup>5</sup>
Mouse	Bone Marrow	MNC	Fresh	0.1-2 x 10 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>
	Spleen	MNC	Fresh	1-10 x 10 <sup>6</sup>	1-10 x 10 <sup>5</sup>
	Fetal liver	MNC	Fresh	0.1-2 x 10 <sup>6</sup>	0.1-2 x 10 <sup>5</sup>

**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, horse, pig, sheep	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 are not cell proliferation assays and do 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 a 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 petri dish usually cast shadows around the outer rim of the petri dish. 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 or contact Preferred Cell Systems™ for advice.

## 10. Recommendations and Tips Prior to Using ColonyGro™.

### (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 ColonyGro™ 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 ColonyGro™

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

### (iii) Dispensing ColonyGro™ Reagent

- Methylcellulose is notoriously difficult to dispense accurately. **DO NOT** use a syringe and needle to dispense any methylcellulose reagent, since it will result in 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
- ColonyGro™ 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 ColonyGro™ 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

### A. 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.

### B. Colony growth is rapid

- Unlike other methylcellulose formulations, ColonyGro™ 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 an 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.

### C. 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 aluminum foil loosely over container or drill holes in the sandwich box lid to allow for gas exchange.

### D. 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](mailto:orders@preferred-cell-systems.com)

Order online at [preferred-cell-systems.com](http://preferred-cell-systems.com)

**Technical Support**

Tel: (719) 264-6251

Email: [info@preferred-cell-systems.com](mailto: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](http://www.preferred-cell-systems.com)

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ColonyGro™ was designed and developed by HemoGenix®

**Preferred Cell Systems™**