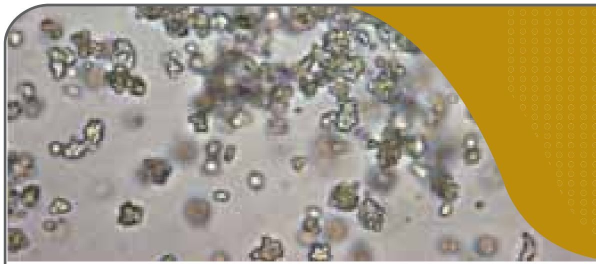


TECHNICAL BULLETIN

MethoCult™ For Rat Hematopoietic Colony Assays

Background

Hematopoietic cell culture assays are used to detect the proliferation and differentiation ability of hematopoietic cells at distinct and successive stages of differentiation, and to measure the frequency of these cells in hematopoietic tissues and purified cell populations. The most common approaches to quantify multi-lineage or single lineage-committed hematopoietic progenitors, called colony-forming cells (CFCs) or colony forming units (CFUs), utilize viscous or semi-solid matrices and culture supplements. These semi-solid culture media promote the proliferation and differentiation of hematopoietic progenitors and allow the clonal progeny of a single progenitor cell to stay together, thus forming a colony of more mature cells. Colony assays are used to quantitate and characterize hematopoietic progenitors from different sources and for the investigation of progenitor responses to growth factors, inhibitors and drugs. Optimized culture media (MethoCult™) are available for the detection of both human and mouse erythroid, monocyte/macrophage, granulocytic, megakaryocytic and multipotent progenitor cells.

Rat models have been used for evaluation of hematopoietic progenitor responses in a number of protocols, including the assessment of drug-induced hematotoxicity,[1] effects of seizures on hematopoiesis,[2] and adeno-associated virus gene transfer into hematopoietic cells.[3] In addition, rats are commonly used as an in vivo model for safety evaluations of new drugs. Several different cytokine combinations have been published to promote rat CFU-GM development.[1,4,5,6] However, formulations of semi-solid colony assay media specifically for rat hematopoietic progenitors have not previously been commercially available. The absence of standardized, reproducible media has prevented the direct comparison of studies performed at different time points or by researchers at various institutions.

MethoCult™ Methylcellulose-Based Medium for Rat CFU-GM Assays

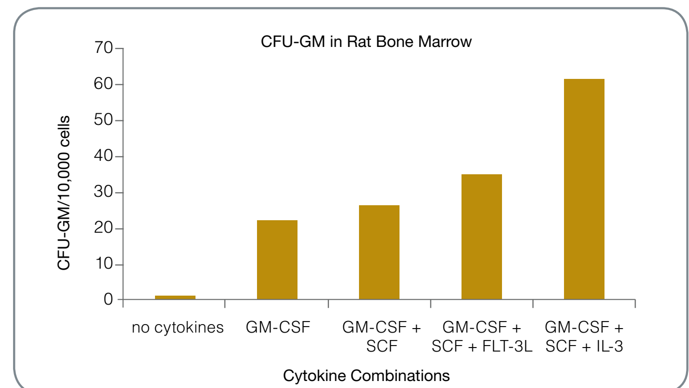
STEMCELL Technologies Inc. has developed a methylcellulose-based MethoCult™ medium that can be used for CFC assays on hematopoietic cells from rat tissues. MethoCult™ media are manufactured using pre-tested components and highly controlled processes in a clean-room facility. Rigorous quality control testing ensures a reproducible and reliable product with minimal lot-to-lot variation. Researchers can be confident that experimental results are dependent on the variables tested and not compromised by inconsistent medium preparations.

Procedure

Rat bone marrow (BM), spleen and peripheral blood (PB) cells were isolated from three- to seven-month-old Noble rats or eleven-week old Sprague-Dawley rats. Nucleated cells were counted according to standard procedures. Duplicate or triplicate cultures containing BM, spleen or PB cells in 1.1 mL of MethoCult™ medium in 35 mm culture dishes were incubated for 9 - 14 days at 37°C, 5% CO_2 in air and >95% humidity. Colony numbers were assessed using an inverted microscope equipped with 2X, 4X and 10X planar objective lenses.

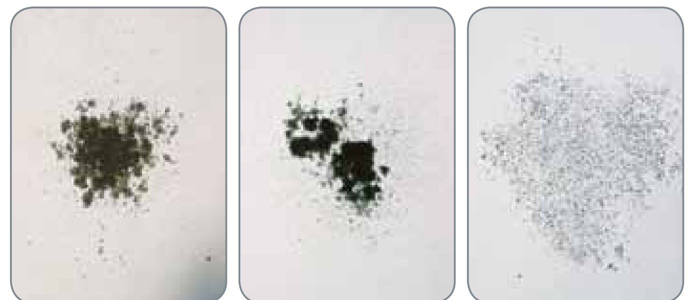
Results

FIGURE 1. Cytokine Responsiveness of CFU-GM in Rat Bone Marrow



The combination of GM-CSF, SCF and IL-3 provides optimal CFU-GM colony numbers from rat bone marrow cells.

FIGURE 2. Examples of Rat CFU-GM-Derived Colonies Cultured in MethoCult™ GF R3774



Photographed at 40X magnification after 11 days of culture.

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MethoCult™ Methylcellulose-Based Medium for Rat Granulocyte/Macrophage Colony-Forming Cell Assays

As shown in Figure 1 the combination of granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF) and interleukin-3 (IL-3) provides optimal growth of rat colony-forming unit-granulocyte, macrophage (CFU-GM), colony-forming unit-granulocyte (CFU-G) and colony-forming unit-macrophage (CFU-M) colonies from rat bone marrow cells. Colonies could be identified and counted after 9 - 11 days of culture.

A complete medium, MethoCult™ GF R3774 (Catalog #03774), containing this cytokine combination is available as a ready-to-use formulation from STEMCELL Technologies. A cytokine-free medium, MethoCult™ Z4003 (Catalog #04003) is also available for testing of cytokines and for testing in vitro drug sensitivity of rat G/M progenitors after the addition of rat GM-CSF (Catalog #02985) or other cytokines chosen by the user.[7,8]

Representative examples of rat CFU-GM-derived colonies cultured in MethoCult™ GF R3774 are shown in Figure 2. The optimal plating density for culture of rat CFU-GM colonies from BM cells is ~1×10^4 to 2×10^4 cells per 35 mm dish (Table 1). For spleen and PB cells, the number of cells plated per 35 mm dish should be at least 1×10^5. Plating spleen and PB cells at two or even three different plating densities, e.g. 1×10^5, 3×10^5 and 1×10^6 per 35mm dish, is recommended to ensure that optimal colony numbers (50 - 100 per dish) are obtained with at least one of the plating densities.

MethoCult™ Methylcellulose-Based Medium for Rat Erythroid Colony-Forming Cell Assays

A complete erythropoietin (EPO)-containing methylcellulose medium, MethoCult™ SF M3436 (Catalog #03436), originally developed for culture of mouse burst-forming unit-erythroid (BFU-E), is also suitable for colony assays of rat erythroid progenitor cells from BM, spleen and PB. As shown in Figure 3, >90% of colonies cultured from rat BM in MethoCult™ SF M3436 were erythroid.

Two types of erythroid colonies were identified: small colonies (~100 - 200 cells per colony) containing several distinct clusters of 10 - 20 erythroblasts (mature BFU-E, Figure 4A) and large colonies (>1000 cells per colony) with a dense core and containing multiple erythroblast clusters (immature BFU-E, Figure 4B). A small proportion of colonies were classified as non-erythroid (Figure 3). These colonies consisted of a single cluster (Figure 4C) or multiple clusters (Figure 4D) of cells that were larger and more distinct from each other than the erythroblasts in the BFU-E colonies and did not show evidence of hemoglobinization. These colonies could not be classified as myeloid, since they did not develop in the absence of EPO (data not shown). This suggested they were derived from immature erythroid progenitors that may require more time than the standard 12 - 14 day culture period for full erythroid differentiation.

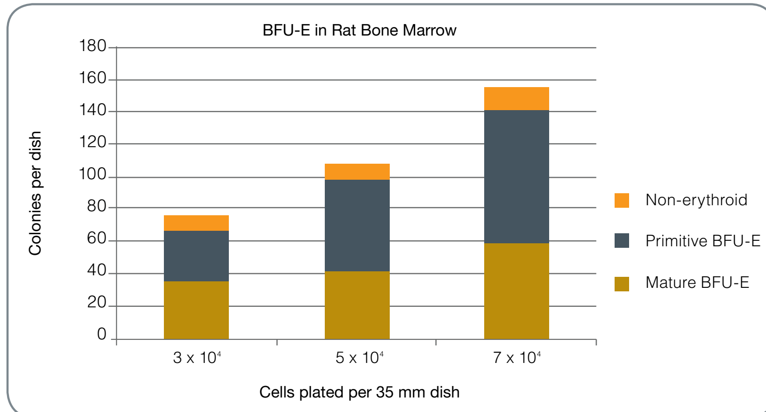
The optimal plating density for culture of erythroid progenitors from rat BM is $2.5 - 5 \times 10^4$ cells per 35 mm dish (Table 1). Erythroid colonies can also be cultured from rat spleen and PB cell preparations, but plating densities need to be higher, i.e. $3 \times 10^5 - 1 \times 10^6$ cells per 35 mm dish (Table 1).

Table 1. Recommended Plating Densities for Rat CFC Assays

CELL SOURCE	PROGENITORS DETECTED	METHOCULT™ FORMULATION	CELLS PER 35 MM DISH
Rat Bone Marrow	CFU-GM	R3774, Z4003	$1 - 2 \times 10^4$
Rat Bone Marrow	BFU-E	M3436	$2.5 - 5 \times 10^4$
Rat Spleen	CFU-GM	R3774, Z4003	$1 - 10 \times 10^5$
Rat Spleen	BFU-E	M3436	$3 - 10 \times 10^5$
Rat Peripheral Blood	CFU-GM	R3774, Z4003	$1 - 10 \times 10^5$
Rat Peripheral Blood	BFU-E	M3436	$3 - 10 \times 10^5$

These plating densities were established using normal healthy Noble and Sprague-Dawley rats. For other strains, transgenic or treated rats and for cytotoxicity testing it is recommended to plate cells at two to three different plating densities .

FIGURE 3. Assay of BFU-E in Rat Bone Marrow

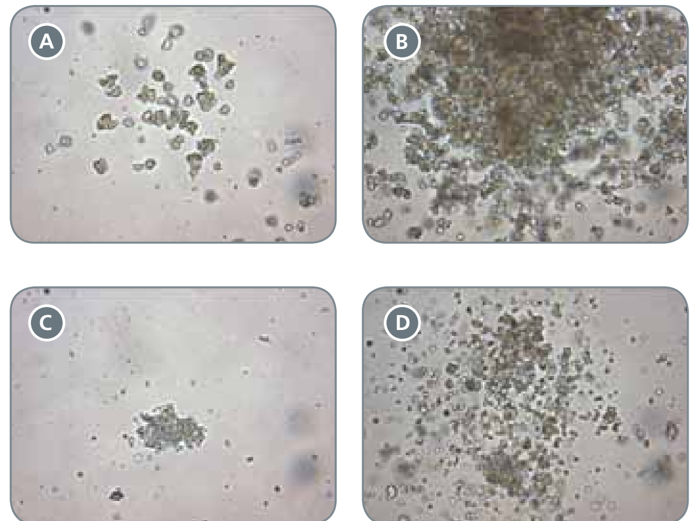


Rat BM cells were plated at the indicated cell concentrations in MethoCult™ SF M3436 and colonies were counted after 14 days of culture. See text for details.

Applications of the Rat CFC Assay

- Testing in vitro drug sensitivity of rat hematopoietic progenitors
- Quantitation of rat hematopoietic progenitors
- Assessing hematopoiesis in transgenic rats
- Optimization of gene transfer protocols into rat cells

FIGURE 4. Examples of colonies cultured from rat BM in MethoCult™ M3436.



A. Mature BFU-E-derived colony; B. Primitive BFU-E-derived colony; C, D. Colonies classified as non-erythroid. Photographed at 20X magnification after 14 days of culture.

Table 2. Product Information

PRODUCT NAME	UNIT SIZE	CATALOG #	PROGENITORS DETECTED
MethoCult™ GF R3774	100 mL	03774	CFU-GM
MethoCult™ Z4003	80 mL	04003	CFU-GM*
MethoCult™ SF M3436	100 mL	03436	BFU-E

*Requires supplementation with rat GM-CSF (Catolog #02985), rat IL-3 (Catalog #02986) and/or other cytokines.

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