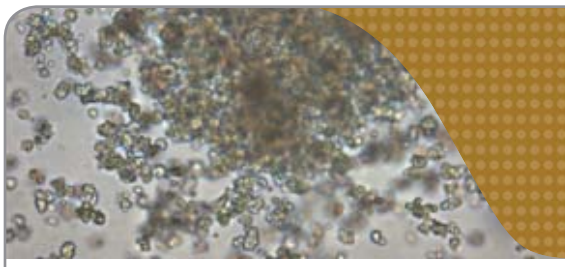

TECHNICAL BULLETIN

MethoCult™ for Rat Hematopoietic Colony-Forming Unit Assays

Background

The colony-forming unit (CFU) assay is an in vitro functional assay used to quantitate and characterize multipotential and lineage-committed hematopoietic progenitor cells, and to investigate their responses to growth factors, inhibitors and drugs. Semi-solid culture media are supplemented with cytokines to promote the proliferation and differentiation of hematopoietic progenitors while the viscocity of the media constrains the progeny of a single progenitor cell, thus forming a colony of mature cells. Optimized culture media (MethoCult™) are available for the detection of erythroid (CFU-E, BFU-E), granulocyte/macrophage (CFU-G, CFU-M, CFU-GM) and multipotent (CFU-GEMM) 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] effect of seizures on hematopoiesis[2] and adeno-associated viral 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]

MethoCult™ Methylcellulose-Based Medium for Rat CFU-GM 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) supports the optimal growth of myeloid colonies derived from rat bone marrow (BM). Colonies could be identified and counted after 9 - 14 days of culture.

A complete medium, MethoCult™ GF R3774 (Catalog #03774), containing this cytokine combination is available as ready-to-use formulation from STEMCELL Technologies.

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

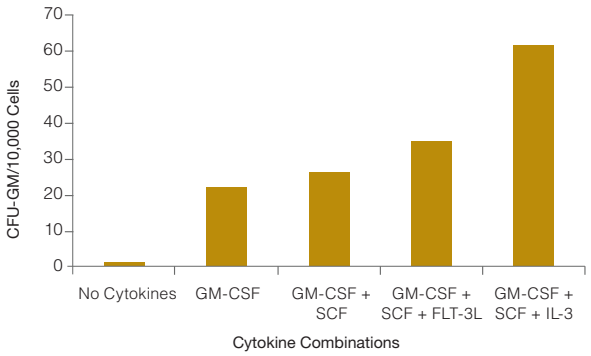

Figure 1. Cytokine Responsiveness of Rat BM-Derived CFU-GM
The combination of GM-CSF, SCF and IL-3 provides optimal CFU-GM colony numbers from rat BM cells.

Figure 2. Rat BM-Derived CFU-GM Colonies in MethoCult™
Photographed at 40X magnification after 11 days of culture in MethoCult™ GF R3774.

MethoCult™ Methylcellulose-Based Medium for Rat BFU-E Assays

A complete erythropoietin (EPO)-containing methylcellulose medium, MethoCult™ SF M3436 (Catalog #03436, Table 1), originally developed for the culture of mouse burst-forming unit-erythroid (BFU-E) progenitors, 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.

Products for Rat CFU Assays

PRODUCT NAME	CATALOG # (SIZE)	PROGENITORS SUPPORTED
MethoCult™ GF R3774	03774 (100 mL)	CFU-G, CFU-M, CFU-GM*
MethoCult™ SF M3436	03436 (100 mL)	BFU-E

*Allows quantitation of total myeloid colonies.

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MethoCult™ for Rat CFU Assays

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, 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 (not shown) 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 9 - 14 day culture period for full erythroid differentiation.

The optimal plating density for culture of erythroid progenitors from rat BM is 2.5 - 5 x 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 x 10^5 - 1 x 10^6 cells per 35 mm dish (Table 1).

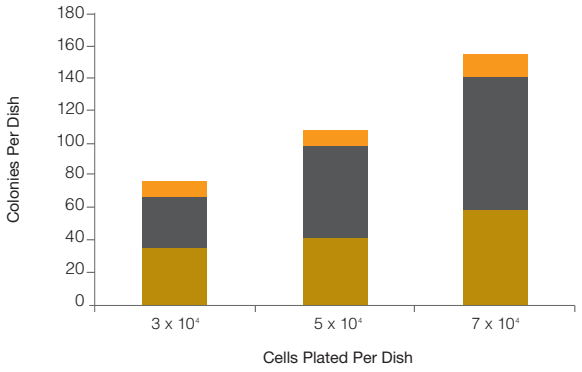

Figure 3. Colony Types in BFU-E Assay of Rat BM with MethoCult™

Rat BM cells were plated in 35 mm dishes at the indicated cell concentrations in MethoCult™ SF M3436 and colonies (orange = non-erythroid, grey = primitive BFU-E, gold = mature BFU-E) were counted after 14 days of culture.

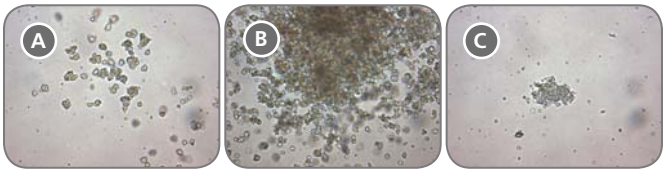

Figure 4. BM-Derived Rat BFU-E Colonies in MethoCult™ SF M3436

(A) Mature BFU-E-derived colony; (B) Primitive BFU-E-derived colony; (C) Non-erythroid colony. Photographed at 20X magnification after 14 days of culture.

Plating Densities for Rat CFU Assays

Rat BM, spleen and PB cells were isolated from 3- to 7-month-old Noble rats or 11-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 R3774 or M3436 medium were plated in 35 mm culture dishes, and incubated for 9 - 14 days at 37°C, 5% CO_2 and > 95% humidity. Colony numbers were assessed using an inverted microscope equipped with 2X, 4X and 10X planar objective lenses. Recommended plating densities for rat CFU assays determined by this procedure are shown in Table 1.

Applications of the Rat CFU Assay

- In vitro model for drug toxicity testing
- Assessing hematopoiesis in transgenic models
- Optimization of gene transfer protocols

Table 1. Recommended Plating Densities for Rat CFU Assays

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

These plating densities were established using healthy Noble and Sprague-Dawley rats. For other strains, transgenic animals or cytotoxicity testing, it is recommended to plate cells at 2 - 3 different plating densities.

References

1. Matsumura-Takeda K et al. (2002) Toxicol In Vitro 16:281-288.
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6. Lautraite S et al. (1997) Cell Biol Toxicol 13:175-183.

For related products for rat HSPC research, including specialized culture media, antibodies and cytokines, visit **www.stemcell.com/HSPCworkflow** or contact us at **techsupport@stemcell.com**.

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