Contract Assay Services

For Stem and Progenitor Cells

STEMCELL™ TECHNOLOGIES

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Helping Make Your Research Work

Contract Assay Services by STEMCELL Technologies Inc. combines the power of quality reagents with the practical knowledge of more than 50 highly qualified scientists to provide customized assays to meet your needs. We have performed more than 500 studies for more than 100 organizations around the world.

Custom-Designed Studies

Introduction

Contract Assay Services works with you to develop and perform flexible custom-designed experiments that meet your unique needs and goals. By harnessing our industry-standard reagents and extensive stem cell biology expertise, you will enhance your ability to determine the potency and safety of compounds earlier in the drug development process, thereby saving valuable time and resources.

Our experts have conducted more than 500 studies and have helped over 100 organizations worldwide with their specific research needs.

Primary Cell Assays for Drug Development

A high failure rate of late stage drug candidates is cost prohibitive. Traditional drug discovery platforms based on animal, tumor or genetically-transformed cell lines are often poorly representative of the human condition. We can help you obtain more clinically-relevant data sooner by using primary progenitor cells. This may significantly improve your drug candidate's translation into the clinic.

Our validated and cell-based assays:

- Determine safety and efficacy of compounds
- Provide clinically relevant, cost-effective information throughout the drug discovery process
- Reduce animal testing

Studies are performed on:

- Hematopoietic stem and progenitor cells
- Immune cells (lymphoid cells)
- Mesenchymal stem and progenitor cells
- Neural stem and progenitor cells

The Highest Level of Service

We are committed to providing you with the highest level of service.

- Confidential consultation with our expert scientific staff
- Custom-designed studies to meet your specific requirements
- Studies performed using STEMCELL Technologies' industry standard reagents manufactured under ISO13485:2003 guidelines
- Thorough and timely reporting of data delivered in a comprehensive report that includes summaries, concise details of experimental design, tabulated data and figures, as well as statistical analysis of the data and photographic records

The Colony-Forming Cell Assay

A commonly used primary cell-based assay is the colony-forming cell (CFC) assay. Progenitor cells, in response to cytokines and supplements in the culture medium, proliferate and differentiate into mature cell types that can be distinguished morphologically. The clonal progeny of each progenitor cell form distinct colonies that can be enumerated. A change in the colony numbers and/or morphology of compound-treated cultures compared to control cultures indicates toxicity, inhibitory or stimulatory effects on hematopoietic,[1-10] mesenchymal[3,9] and neural progenitor cells.

Benefits of the CFC assay:

- Yields clinically predictive information, allowing for better planning and a reduction in in vivo studies[2]
- Uses physiologically relevant primary cells
- Fast turnaround time for results with one- to two-week culture period
- Assesses both proliferation and differentiation simultaneously
- Is both quantitative (colony number and size of colonies) and qualitative (cell and colony morphology)
- Can determine both IC_{50} and IC_{90} values

Validation of the CFC Assay

CFC assays for myeloid progenitors have been validated for the determination of maximum tolerated doses (MTD) by the European Centre for the Validation of Alternative Methods (ECVAM). Assays for colony-forming unit - granulocyte, macrophage (CFU-GM) and colony-forming unit - megakaryocyte (CFU-Mk) have been shown to be predictive of clinical outcomes such as neutropenia[2,6] and thrombocytopenia.[7]

Hematopoietic Stem and Progenitor Cell Assays

The hematopoietic CFC assay can be used for the following applications:

- Screening for toxic effects of compounds in blood or bone marrow
- Determining whether small molecule compounds have inhibitory or stimulatory effects
- Assessing clinical samples
- Evaluating myelotoxicity as a first step in immunosuppression testing

Toxicity, Inhibition and Stimulation

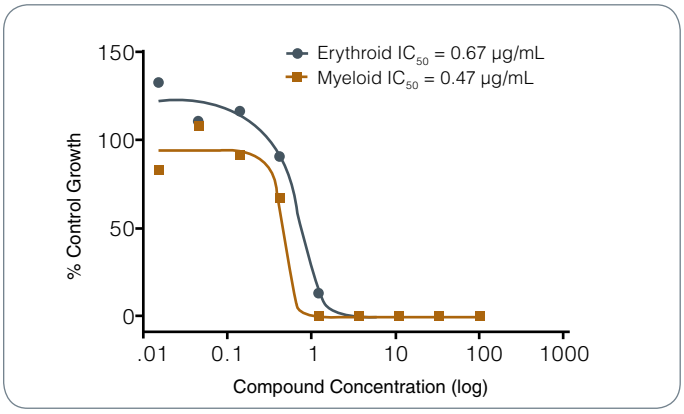
The CFC assay can be used to evaluate the toxicity and inhibition, or stimulation of compounds on hematopoiesis. Because the CFC assay is both qualitative and quantitative, the effects of compounds are determined by changes in the number of colonies and/or changes in colony size.

By altering the compound concentration or combination and measuring the effects on colony formation, IC_{50} and IC_{90} values for toxicity and EC_{50} values for stimulation can be determined.

Determining the effects of compounds on hematopoiesis can have important clinical implications. For example, toxicity or inhibition of hematopoiesis can lead to neutropenia, anemia and thrombocytopenia.

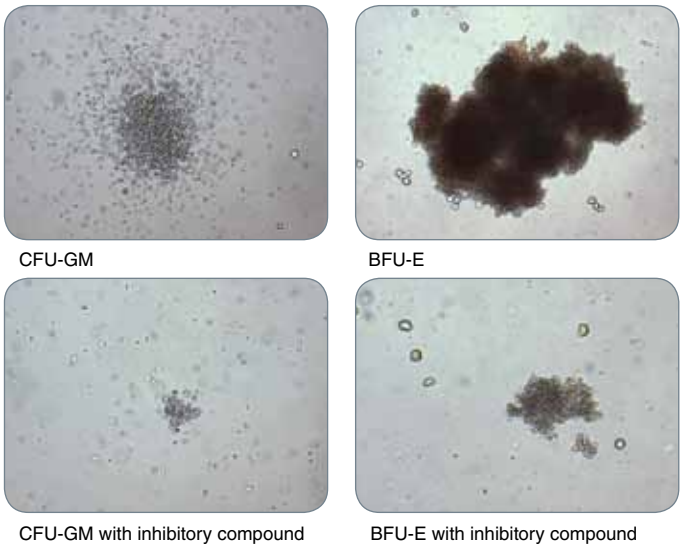
Figures 1 and 2 show CFC assay data for hematopoietic inhibition. Figure 3 shows CFC assay data for stimulation.

FIGURE 1. Determination of IC_{50} values for 5-Fluorouracil



Dose response curves and IC_{50} values for both human BM-derived erythroid and myeloid progenitors incubated with 5-Fluorouracil.

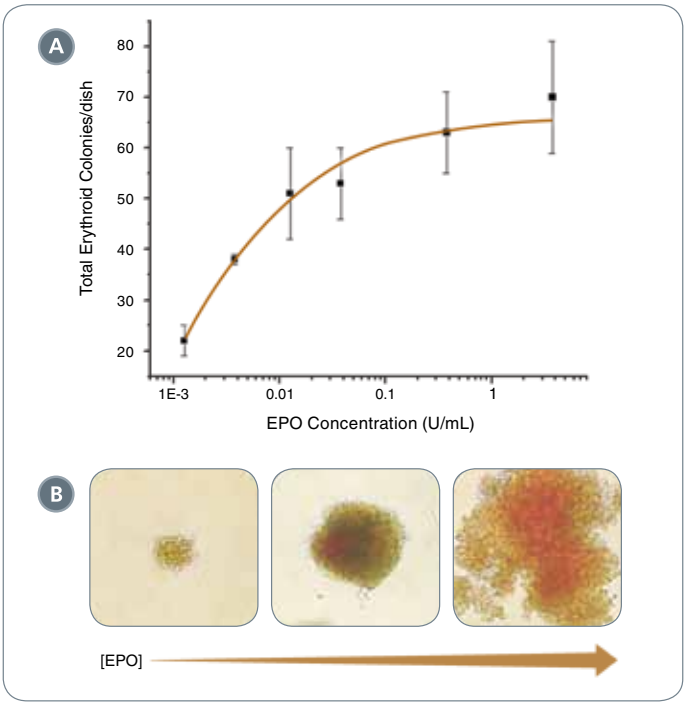
FIGURE 2. Colony size changes in the presence of an inhibitory compound



CFU-GM | BFU-E

CFU-GM with inhibitory compound | BFU-E with inhibitory compound

FIGURE 3. Stimulation by erythropoietin (EPO)



Stimulation of erythroid progenitor proliferation by erythropoietin (EPO) is both quantifiable (A) and qualitative (B, colony size increases with increasing EPO). In vivo models can be used to confirm stimulatory activity of molecules.

Species-Specific Data

There can be significant differences in hematosensitivity to pharmaceuticals and toxins between species. Performing the CFC assay with primary cells from different species can assist in determining the animal model most representative of the human condition for a particular class of compound. Contract Assay Services can perform CFC assays on human, mouse, rat and canine cells.

TABLE 1. CFU-GM IC_{50} values for compounds in different species

COMPOUNDS	HUMAN	MOUSE	CANINE	RAT
Topoisomerase				
Topotecan	**7.69 nM**	168.80 nM	**4.40 nM**	30.96 nM
Irinotecan	**288.10 nM**	>1000 nM	**358.10 nM**	>1000 nM
Camptothecin	**1.03 nM**	6.16 nM	**0.75 nM**	9.16 nM
Anti-Proliferative				
Doxorubicin	**0.03 µM**	**0.01 µM**	0.002 µM	0.006 µM
Cisplatin	**4.21 µM**	**6.79 µM**	0.97 µM	2.68 µM
5-Fluorouracil	**3.84 µM**	**3.08 µM**	0.23 µM	1.62 µM
Tyrosine Kinase Inhibitors				
Sunitinib	**0.08 µM**	1.10 µM	**0.01 µM**	0.22 µM
Imatinib	**2.16 µM**	>30 µM	**1.99 µM**	>30 µM
Erlotinib	**15.27 µM**	19.39 µM	**10.36 µM**	34.67 µM
Environmental Toxin				
Lead Nitrate	**0.98 µM**	2.05 mM	0.04 mM	**1.20 mM**

Immunomodulation

Assessment of myelotoxicity is recommended as a first step in immunosuppression testing. This can be done using the CFC assay (see page 4 for more information).

> *"Compounds that are capable of damaging or destroying the bone marrow will often have a profoundly immunotoxic effect, since the effectors of the immune system itself will no longer be available. Thus, if a compound is myelotoxic, there may be no need to proceed with additional evaluation since the material will be a de facto immunotoxicant…An initial evaluation of myelotoxicity should be performed. If a compound is myelotoxic, there may be no need to proceed with additional evaluation."* [11]

If a compound is not myelotoxic and further immunosuppression testing is required, Contract Assay Services can assess various immune system components using custom in vitro studies.

Cells from primary human or mouse sources are isolated, enriched and assayed to assess in vitro effects of test articles on immune pathways, including pro- or anti-inflammatory responses. Custom assays are designed and optimized to fit your particular question using the following services:

- FACS analysis
- Quantification of immune effector molecules (e.g. cytokines and immunoglobulins) using:
  - ELISA
  - Cytometric bead analysis (for simultaneous analysis of multiple analytes)
- Cell proliferation (BrdU protocol)
- Chemotactic assays
- Enrichment and purification

Assessment of Clinical Samples

Contract Assay Services tests hematopoietic clinical samples, including assessing progenitor content and characteristics. Examples of samples include:

- Peripheral blood samples from clinical trials
- Mobilized peripheral blood samples for transplantation
- Cord blood samples for banking and/or transplantation

$CD34^+$ cells are measured in clinical samples and the result is used in conjunction with progenitor content and characteristics to provide an overall assessment of the sample. Contract Assay Services quantifies $CD34^+$ cells using the ISHAGE protocol.

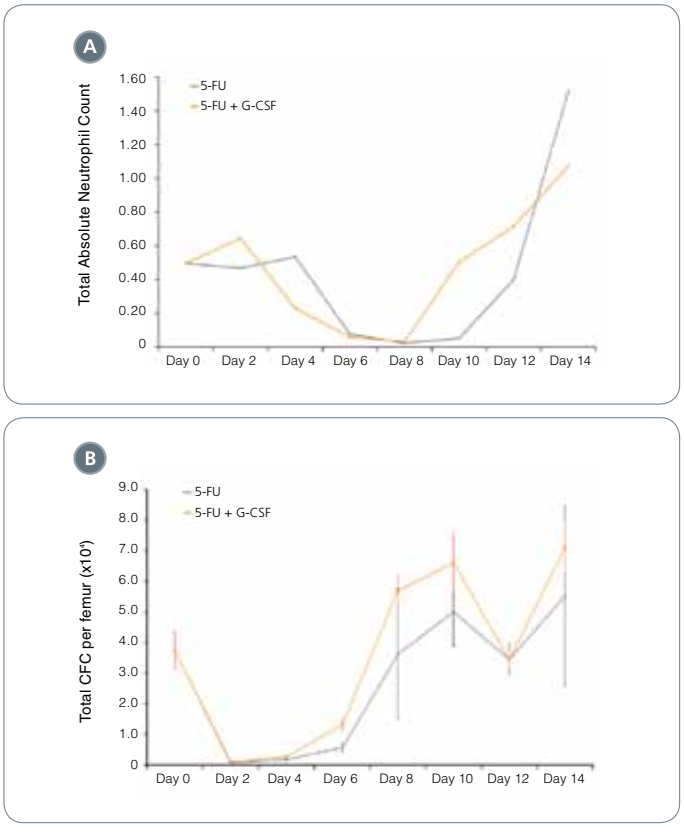
In Vivo Hematopoietic Assays

In vivo assays are also used to answer questions about stimulation of hematopoiesis. Examples include:

- Mobilization of hematopoietic stem cells into the peripheral blood
- Progenitor frequency evaluation in transgenic or knockout mice
- Engraftment potential of hematopoietic stem cells in congenic mice recipients
- Stimulatory effects of compounds on human hematopoietic stem cells using the NOD/SCID mouse model

Figure 4 shows an example of the use of an in vivo assay to determine hematopoietic recovery after treatment with an inhibitory compound.

FIGURE 4. Recovery of CFC after injection of 5-Fluorouracil with and without G-CSF pre-treatment



Wild type BalbC mice were treated with 5-Fluorouracil on day 0. Animals treated with G-CSF (orange line) showed faster recovery of neutrophils in the blood (Figure A) and total progenitors (Figure B) in the bone marrow.

Mesenchymal Stem and Progenitor Cell Assays

Mesenchymal stem cells, under the appropriate conditions, can differentiate into cells that make up adipose tissue, cartilage, bone and muscle.

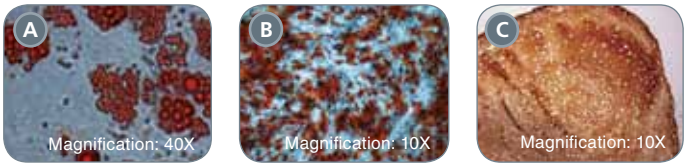
Expansion and Characterization

Contract Assay Services expands mesenchymal cells and characterizes them based on the International Society for Cellular Therapy (ISCT) guidelines.[12,13]

Criteria for human mesenchymal stem cells include:

- Ability to adhere to tissue culture plastic
- Be positive for CD105, CD73 and CD90
- Be negative for CD45, CD34, CD14 or CD11b, CD79a, CD19 and HLA-DR
- Ability to differentiate to osteoblasts, adipocytes and chondroblasts (Figure 5) under standard in vitro differentiating conditions

FIGURE 5. Human bone marrow-derived MSCs cultured in MesenCult™-XF display multi-lineage differentiation potential.



A. Oil red O staining of adipocytes generated from passage 1 MSCs.
B. Alizarin Red detection of Ca^{++} deposits indicates the formation of bone structures in cells generated from passage 4 MSCs.
C. Collagen II staining of chondrocytes generated from passage 2 MSCs.

Toxicity, Inhibition and Stimulation

The functional colony-forming unit - fibroblast (CFU-F) assay quantifies mesenchymal stem and progenitor cells from human bone marrow or mouse compact bone. See page 4 for more information about colony-forming assays and their benefits.

CFU-F assays are used to quantify and qualitatively assess the effects of compounds on mesenchymal stem cells, including:

- Frequency and proliferative potential
- Overall expansion potential
- Differentiation potential into adipose, osteogenic and chondrocyte lineages

FIGURE 6. Effects of doxorubicin on Human CFU-F

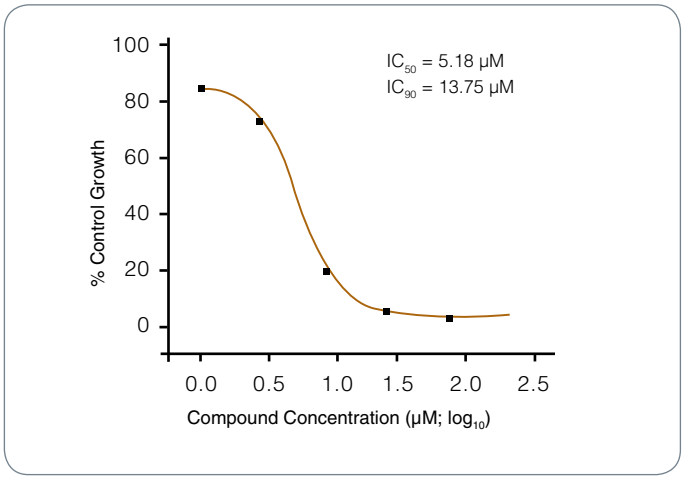
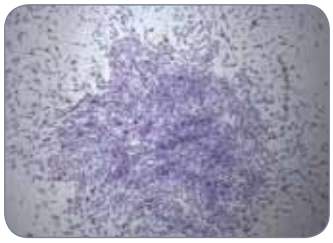
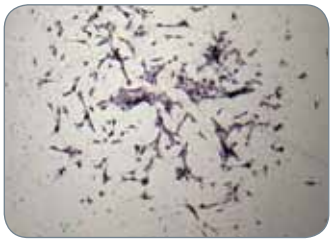


FIGURE 7. Human bone marrow-derived CFU-F colonies in the presence of an inhibitory compound



Control human CFU-F



Human CFU-F with inhibitory compound

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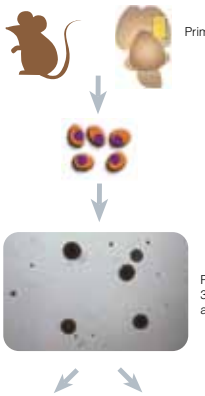
Neural Stem and Progenitor Cell Assays

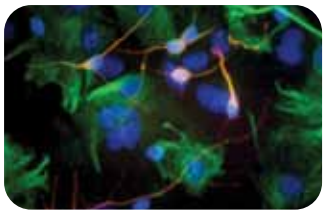
Toxicity and Differentiation

Contract Assay Services determines the effects of compounds and environmental toxins on the proliferation of primary neural stem and progenitor cells using cell-based assays, and on the ability of primary neural stem and progenitor cells to differentiate into neurons, astrocytes and oligodendrocytes.

FIGURE 8. Assays for neural stem and progenitor cells

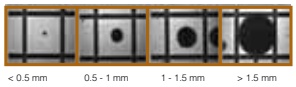


Differentiation assays are used to assess the effects of your compound on the potential of neural stem and progenitor cells to differentiate into astrocytes, neurons and oligodendrocytes.

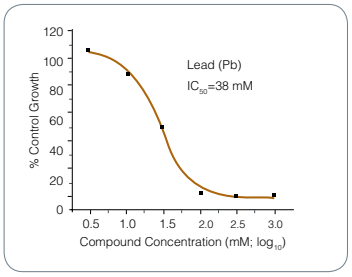


Mouse neurospheres were dissociated and induced to differentiate. Neurons (red) and astrocytes (green) can be detected using immunofluorescent methods.

Proliferation potential can be quantified using the NCFC Assay.



Larger NCFC colonies (>1.5 mm in size) are formed by neural stem cells; the smaller NCFC colonies are formed by more mature neural progenitors.



Dose response curve and IC_{50} for lead in the NCFC Assay

Sphere Assays

Liquid sphere assays are used to grow and assess certain stem cells. Single cell suspensions are cultured in a specialized medium that allows cells to form clusters of cells, or spheres, which are made up of primitive, undifferentiated cells.

Contract Assay Services perfoms sphere assays using optimized media from STEMCELL Technologies, including NeuroCult™ and MammoCult™. The applications of the sphere assay vary and it is customizable.

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