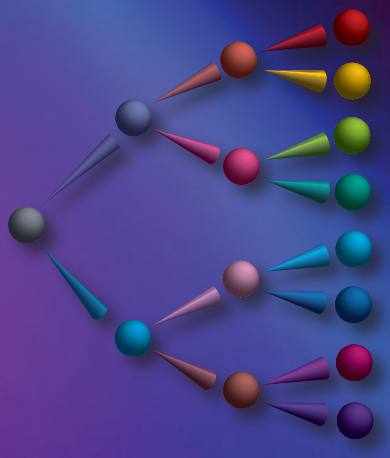

HemoGenix®

Quality Control,
Stem Cell Potency
and
Patient Monitoring
Assay Kits
for the
Stem Cell Transplantation
and
Cord Blood Bank
Processing Laboratory

The Company that brought

Stem Cell Transplantation and Cord Blood Quality Control and Stem Cell Potency Assays into the 21st Century

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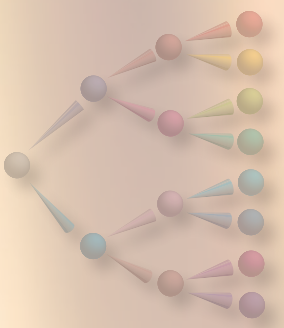
www.hemogenix.com

New Technology Helps Reduce Risk and Improve Outcome for the Patient

Stem cell transplantation has been part of the clinical community since the mid 1970s. Starting with bone marrow transplantation followed by the use of mobilized peripheral blood when recombinant G-CSF was introduced in 1987 and then with umbilical cord blood in 1989, procedures and regimen for treating patients with life-threatening hematological disorders have improved dramatically. New regulatory guidelines require ever-stricter improvements in most aspects of the stem cell transplantation process. Instrumentation has significantly improved both in sensitivity and in sophistication. Yet, the basic tests and assays required to determine the "quality" of the product prior to transplantation have remained unchanged for decades.

The exception has been HLA typing. However, this highly advanced technology is coupled with four parameters that, at best, provide limited correlative information to reduce risk and improve outcome for the patient. The total nucleated cell (TNC) count is usually used to determine the number of cells that need to be transplanted. But TNC contain cells that do not proliferate and mask the mononuclear cell fraction which contain the stem cells. Viability is performed by dye exclusion that detects membrane integrity, but provides no information on cellular and mitochondrial integrity indicative of cell functionality. Although other membrane markers for stem cells are now being used, CD34 membrane expression, is still thought of as a "stem cell marker". However, lineage-specific progenitor cells, which have no stem cell characteristics, also express this antigen. Despite this, TNC, viability and CD34 enumeration can be standardized and validated. In contrast, the colony-forming cell (CFC) assay, which has been employed in the field since 1971, is so subjective, it has never been possible to validate as a quality control assay, let alone a potency assay required by regulatory agencies.

HemoGenix® has been the leader in hemotoxicity testing since it was founded in 2000. HemoGenix® has also been proactive and innovative in developing a family of quality control, cell potency and patient monitoring assays specific for the processing laboratory that are not only aimed at regulatory compliance by being standardized and validated, but are also rapid and easy to use.

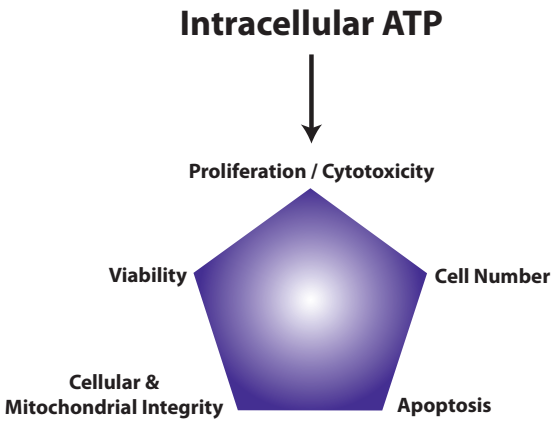


HemoGenix®

The Concept of Measuring Proliferation Potential and Cellular Functionality for Stem Cell Transplantation Products.

For a stem cell transplant to be successful, the stem cells must proliferate. HemoGenix® was the first to introduce the concept of measuring a single biochemical marker, namely intracellular ATP (iATP), to measure proliferation potential, cellular functionality and viability as a means to establish quality control and potency of a stem cell product destined for transplantation.

ATP - A Powerful Biochemical Marker of Cellular Function

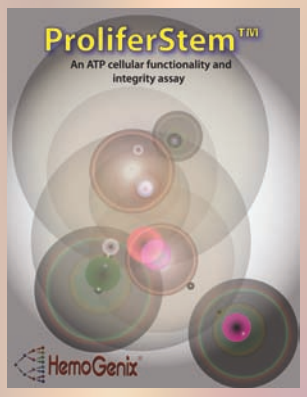


ProliferStem™ - A cell functionality assay for pre-cryo and post-thaw cord blood, mobilized peripheral blood and fresh bone marrow stem cell products.

To distinguish stem cell products that have a high probability of proliferating and growing from those that do not, HemoGenix® developed ProliferStem™. ProliferStem™ is used at the same time as TNC/MNC counts, viability and CD34 are measured.

ProliferStem™ is an iATP assay. No cell culture is required. It can be used to determine whether a cord blood unit should be cryopreserved or rejected and if a cord blood unit will exhibit cellular functionality after it has been thawed. Cellular functionality can also be measured on mobilized peripheral blood and bone marrow. Cell functionality is the ability of cells to produce cellular energy in the form of iATP. If iATP is produced, the cells demonstrate a functional cellular and mitochondrial integrity and they would be expected to grow in both HALO®-96 SPC-QC and HALO®-96 PQR assays. Like all HALO® assays, ProliferStem™ incorporates an ATP standard and controls allowing calibration, standardization and validation of the assay.

Use ProliferStem™ to determine iATP concentrations and you determine cellular and mitochondrial integrity, viability and proliferation potential, all in one easy, 30 minute assay.



ProliferStem®

to measure cellular functionality of pre-cryo and post-thaw cord blood, mobilized peripheral blood and fresh bone marrow.

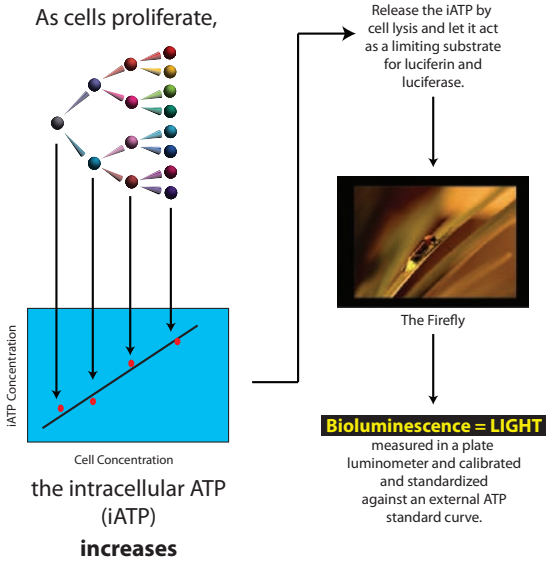
Stem Cell Proliferation, Viability, Cell Dose and Cellular and Mitochondrial Integrity are all integrated into a single assay

The Halo® Principle

In 2002, HemoGenix® introduced the HALO® Platform, as a replacement for many applications that use the CFC assay. These applications include, quality control, stem cell potency measurements and patient monitoring of lympho-hematopoiesis. All of these applications require a standardized and validated assay that can be compliant with regulatory guidelines from both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). The family of HALO® assays described in this brochure accomplish this goal.

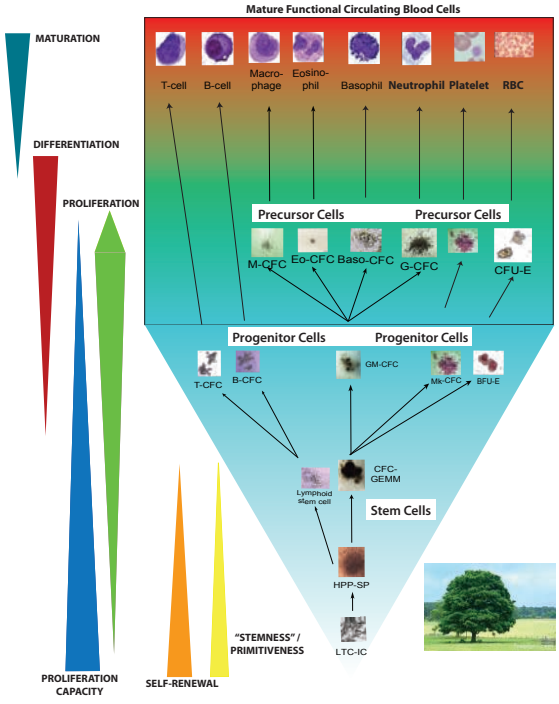
HALO® is an intracellular ATP (iATP)-based bioluminescence proliferation assay performed on cultuted cells. The HALO® Principle is shown in the diagram below.

The HALO® Principle



Since the results obtained from all HALO® assays have been shown to be equivalent to those of the CFC assay (see diagram on page 7), HALO® can detect and measure the proliferation status of all primitive and mature stem cell populations and early lympho- and hematopoietic progenitor cell populations as depicted in the organizational and hierarchical diagram of the lympho-hematopoietic system. In addition, the characteristics of stem cells play an especially important role in the HALO®-96 PQR assay to measure stem cell potency and define release criteria for cord blood units.

THE ORGANIZATION AND HIERARCHICAL TREE OF THE BLOOD-FORMING SYSTEM



Which HALO® Format is

Right for Your Application

	CFC Assay (CAMEO-4)	HALO®-96 SPC-QC	HALO®-96 PQR	HALO®-96 PMT
	Colony-forming cell assay	Stem and Progenitor Cell – Quality Control	Potency, Quality, Release	Patient Monitoring after Transplantation
Type of Assay:	Differentiation	Proliferation	Proliferation	Proliferation
Type of Culture:	Methylcellulose	Methylcellulose or Suspension	Suspension	Methylcellulose or Suspension
Cell Growth:	Clonal	Clonal or Expansion	Expansion	Clonal or Expansion
Parameter measured:	No. of colonies	Intracellular ATP	Intracellular ATP	Intracellular ATP
Method of Readout	Microscopy	Luminometer	Luminometer	Luminometer
Subjectivity:	Subjective	Non-subjective	Non-subjective	Non-subjective
Standardization:	None	External ATP	External ATP	External ATP
Controls:	None	High & low	High & low	High & low
Validated:	No	Yes	Yes	Yes
Format:	4-well 35mm or 35mm Petri dish	96-well plate	96-well plate	96-well plate
Volume of assay:	100µl/well or 1ml/dish	100µl/well	100µl/well	100µl/well
No. of Replicates:	2 to 4	6	6-8	6
Cell Incubation time	14 days	5 or 7 days	5 days	5 or 7 days
Processing/Readout time:	None/~5-10min per dish	~20min/5min per plate	~20min/5min per plate	~20min/5min per plate
Readout Quantitation	No. of colonies / No. of Plated Cells	µM iATP / No. of Plated Cells	µM iATP / No. of Plated Cells	µM iATP / No. of Plated Cells
Training	6-12 months	2 days	2 days	2 days
Proficiency testing	No	Possible	Possible	Possible

HALO®-96 SPC-QC

A standardized and validated routine Stem and Progenitor Cell – Quality Control assay for bone marrow, mobilized peripheral blood and umbilical cord blood processing laboratories

For years, investigators, standards organizations and regulatory agencies have been in a "love, hate relationship" with the CFC assay. On the one hand, it has been the only assay available to detect early progenitor cells and stem cells since 1966 when the method was first published and then adapted for human cells in 1971 by Pike and Robinson. On the other hand, those who use the CFC assay complain about its subjectivity, setup time, difficulty in enumerating colonies under a microscope and the overall lack of consensus not only within, but also between laboratories. Lack of reproducibility means that the CFC assay has never been validated for this application.

What is Standardization and Validation?

Those who propagate the CFC assay for clinical use maintain that the assay is standardized. They point to "standardized" and "consistent" reagents as evidence. Yet standardization is more than ensuring that reagent batches are similar and that they work properly. Assay standardization is defined as the process of checking or adjusting, by comparison with a known standard, the accuracy of the test. Until the development of CAMEO™-96 STD (see page 11), there was no known external standard to which the CFC assay could be compared.

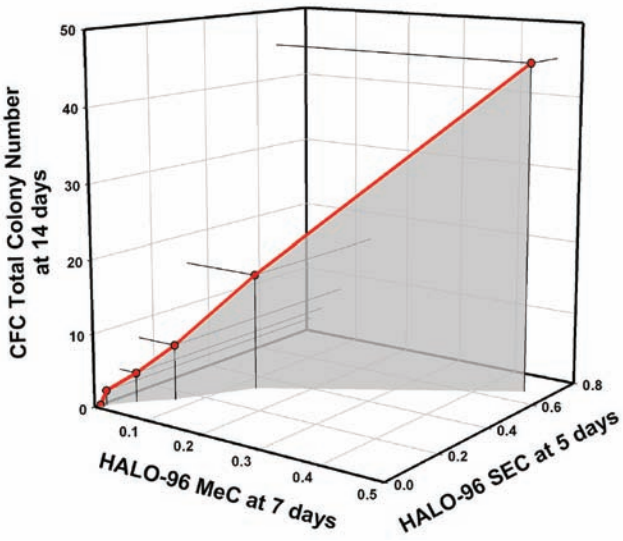
Validation is defined as establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Part of the assay validation procedure involves quantitatively demonstrating accuracy, selectivity, specificity, reliability, reproducibility and robustness. The CFC assay fails miserably for accuracy, reliability, reproducibility and robustness.

An assay that is not calibrated, standardized and validated and is used, in most cases, as a "growth – no-growth" assay, has no place in a clinical setting.

But, there is a solution

HALO®-96 SPC-QC.

The diagram below demonstrates that the results from both HALO®-96 MeC and HALO®-96 SEC correlate directly with total colony counts produced by the CFC assay as a function of cell concentration. This means that both HALO® formats can replace the CFC assay.



In addition, Reems et al (Transfusion, 2008, 48:)620-628) have also shown a correlation (R = 0.89) of HALO®-96 MeC performed in two geographically separate laboratories. They also demonstrated a correlation between HALO®-96 MeC and the total colony counts using the CFC assay.

HALO®-96 MeC versus HALO®-96 SEC

Although HALO®-96 MeC is based on the CFC assay and is therefore a clonal assay, HALO®-96 SEC uses Suspension Expansion Culture technology and has several advantages over the methylcellulose assay format.

- Easier to use
- Greater accuracy dispensing reagents resulting in CVs of 12% or less.
- Increased cell interaction resulting in a shorter lag time to initiate proliferation with a concomitant shorter incubation time of only 5 days (compared to 7 days for HALO®-96 MeC).
- Increased cell interaction also results in a 2-fold increase in sensitivity.

HALO®-96 SPC-QC

The Stem and Progenitor Cell – Quality Control assay was specifically developed to routinely assess the proliferation potential of bone marrow, mobilized peripheral blood or umbilical cord blood in the busy cell processing laboratory. HALO®-96 SPC-QC is only available to measure either the mature hematopoietic stem cell population, CFC-GEMM, or both the CFC-GEMM and the more primitive lympho-hematopoietic stem cell population, HPP-SP, since these are the cell populations upon which stem cell transplantation relies. The CFC-GEMM cell population will provide an excellent indication for potential short-term engraftment, while measuring both mature and primitive stem cell populations will provide information on both short- and long-term engraftment potential without having to use the more laborious and costly LTC-IC assay.

HALO®-96 MeC SPC-QC and HALO®-96 SEC SPC-QC

Choose between 2 different formats, either with or without methylcellulose, both of which have been validated against the CFC assay

HALO®-96 PQR

(Potency, Quality, Release)

The only stem cell potency and release assay that is compliant with regulatory guidance

Regulatory Requirements

In the United States, potency is defined in the Code of Federal Regulations (21 CFR 600.3) as "the specific ability or capacity of a product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result". Potency is defined by the European Medicines Agency (EMEA) as a "quantitative measure of biological activity". A test for potency has also been defined in the U.S. Code of Federal Regulations (21 CFR 610.10), but the EMEA's definition is probably easier to understand as an *in vivo* or *in vitro* test that is "appropriately validated" and "based on a defined biological effect as close as possible to the mechanism(s) of action / clinical response".

Potency measurements have been used for decades to determine the activity and therefore the administration dose of a drug, vaccine, growth factor etc. Potency measurements are now being required for cellular therapy products because:

- It ensures consistency during production/manufacture of biological products.
- It shows product stability.
- It predicts product performance and assurance.
- It allows evaluation and/or correlation with the clinical response.
- It avoids product failure due to toxicity or improper potency.

What is Needed to Measure Potency?

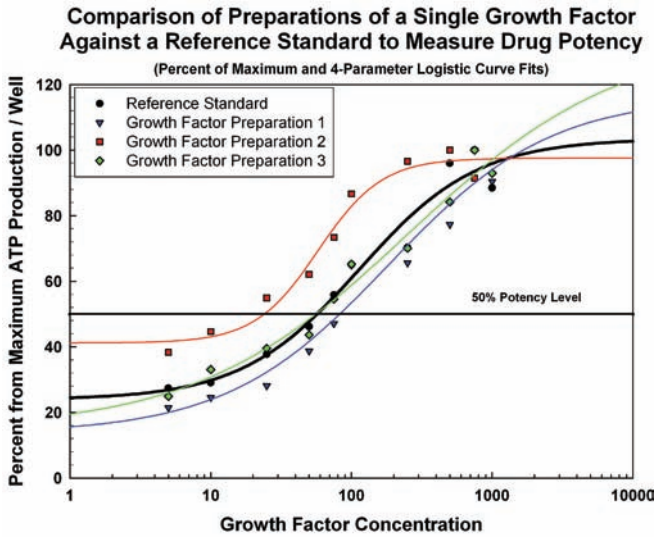
There are three basic requirements for measuring potency. These are:

1. An "appropriate validated" assay.
2. A reference standard (RS) of the same material.
3. A dose response relationship.

As shown in the diagram below, 3 growth factor samples are compared to the same growth factor reference standard to produce statistically parallel dose responses for the linear portion of curves. The horizontal displacement to the right indicates a lower potency ratio than the RS, while a displacement to the left indicates a higher potency ratio and therefore greater activity than the RS.

Can Present Tests or Assays be used to Measure Cell Potency?

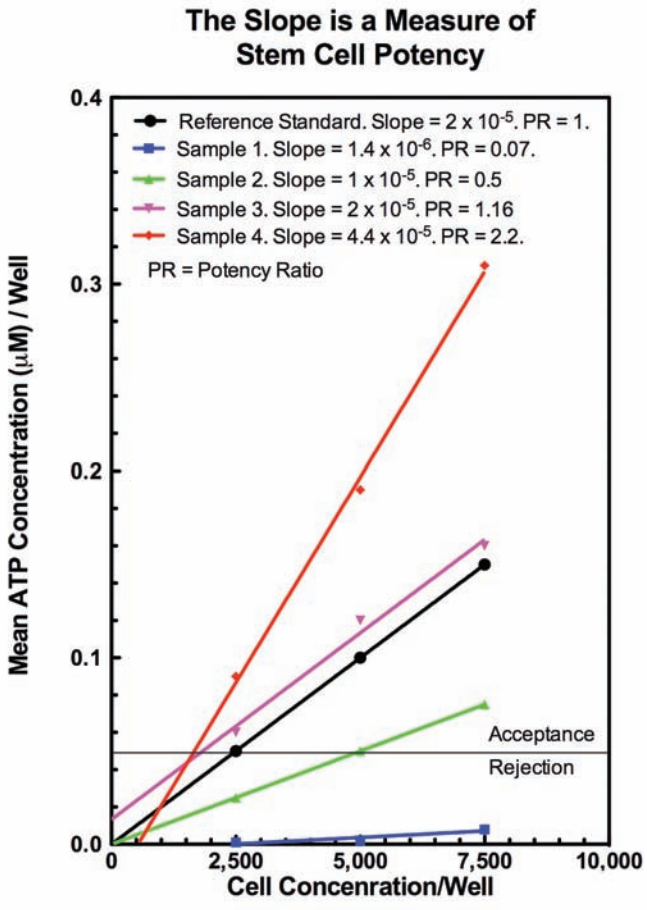
The cellular product and its intended use will define the potency assay. For bone marrow, mobilized peripheral blood or umbilical cord blood stem cell transplantation, the intended use is to effect engraftment and repopulation. This, in turn, requires the cells to proliferate. Since cell number, viability and CD34 antigen expression do not measure proliferation potential, these are not appropriate assays. In addition, since the CFC assay measures differentiation, not proliferation, and is not a validated assay and cannot be validated against any other assay except HALO®, it cannot be used as a potency assay.



How Does HALO®-96 PQR Measure Stem Cell Potency?

The three requirements stated above are all met in HALO®-96 PQR. The assay is certainly "appropriate" because it measures proliferation as a function of intracellular ATP concentration. HALO®-96 PQR is also a "validated" assay. HALO®-96 PQR assay kits contain a vial of cryopreserved umbilical cord blood as reference standard. Tubes containing pre-dispensed culture reagents for either CFC-GEMM or CFC-GEMM and HPP-SP cells populations to measure a 3-point cell dose response for the reference standard and samples are provided. In addition, an ATP standard, high and low controls, ATP monitoring reagent and 96-well plates are also included. In fact, all the reagents and supplies are provided. Just prepare the appropriate cell concentrations, add the correct volume to the tubes and dispense 8 replicate wells. Incubate the cells for 5 days and measure the luminescence of the samples after performing the ATP standard curve. Convert the luminescence readout in RLU to ATP concentrations using the ATP standard curve and plot the linear regressions.

Results such as those shown in the diagram below will be obtained.



The stem cell potency ratio is then determined either by the parallel displacement of the sample to the reference standard (as shown on page 8), or from the difference in slope to the reference standard. Defining release criteria will depend initially on the processing laboratories to ascertain the intracellular ATP concentration below which a sample would be rejected. At HemoGenix®, this value is presently at about 0.05µM iATP/5,000 cells.

HALO®-96 PQR

The only potency assay available to measure the intended use of lympho-hematopoietic stem cell for transplantation

HALO®-96 PMT

(Patient Monitoring after Transplantation)

Are the Transplanted Cells actually Repopulating the Patient?

Whereas HALO®-96 SPC-QC and HALO®-96 PQR were developed to assess the "quality" and potency of the stem cell product prior to transplantation and may even predict engraftment potential, HALO®-96 PMT is used to monitor reconstitution of the patient after transplantation.

Although an assay may be predictive based on results, the disease, conditioning regimen and many other factors can greatly influence engraftment and reconstitution of the patient. During the early phase after transplantation, hematopoietic reconstitution is of prime importance. At later times, lymphopoietic reconstitution is important in order to try and predict lymphoid involvement. Reconstitution may also be "unbalanced" in that cells of one or more lineages may be produced either preferentially or at levels that cannot sustain the patient.

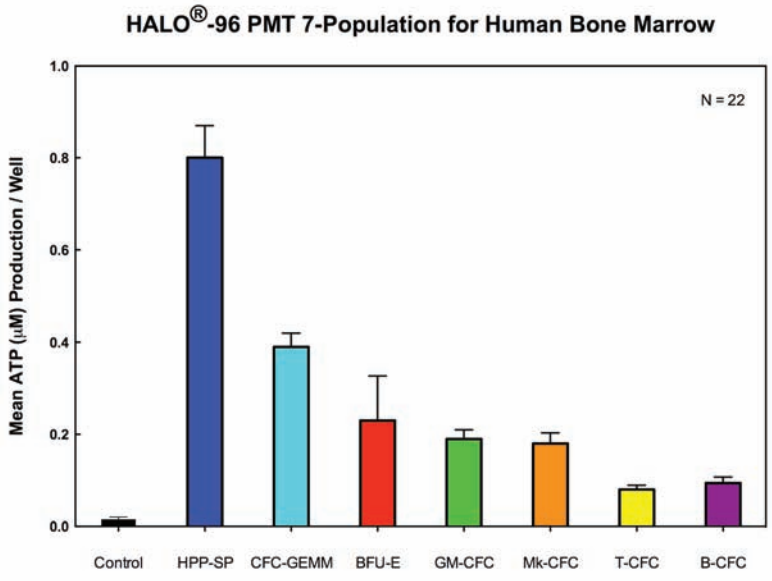
HALO®-96 PMT can help monitor the lympho-hematopoietic status of the patient after transplantation using peripheral blood or a bone marrow sample of less than 500µl.

Like HALO®-96 SPC-QC, HALO®-96 PMT is also available to culture cells either under clonal conditions in methyl cellulose, or using Suspension Expansion Culture (SEC) technology. Unlike HALO®-96 SPC-QC however, HALO®-96 PMT is designed to detect and measure either 4 or 7 cell populations simultaneously. The cell populations detected are:

- 4-Population Assay. CFC-GEMM, BFU-E, GM-CFC and Mk-CFC.
- 7-Population Assay. HPP-SP, CFC-GEMM, BFU-E, GM-CFC, Mk-CFC, T-CFC and B-CFC with a background control.

The 4-population assay can monitor hematopoietic reconstitution, while the 7-population assay provides a "global" picture of both the hematopoietic and lymphopoietic repopulation.

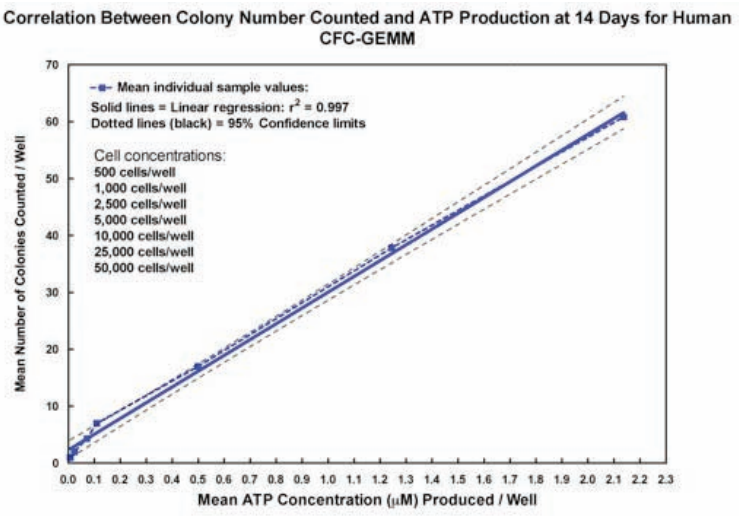
The diagram below shows typical results from normal bone marrow showing expected proliferation potential of all 7 cell populations.



How To Standardize the Colony-Forming Cell (CFC) Assay

Having reagents that show batch consistency does not mean that the assay is standardized. Furthermore, training personnel to count similar types and numbers of colonies produced in the CFC assay also does not mean that the assay is standardized. In order to standardize the CFC assay, it has to be calibrated against an external standard. Since no external standard exists to which the CFC assay can be calibrated, it can not be standardized.

This problem has been solved by HemoGenix®, which has developed CAMEO®-96 STD for stem cell processing laboratories. The assumption that allows CAMEO®-96 STD to be used to standardize the CFC assay is that target cells are cultured using the same reagents under exactly the same conditions. CAMEO®-96 STD combines a 14 day CFC differentiation assay for human bone marrow, mobilized peripheral blood or umbilical cord blood cells, cultured in methylcellulose in a 96-well plate, with an 14 day ATP bioluminescence proliferation assay performed on the same cells in the same plate. After the 14 day incubation period has elapsed, the total number colonies in each well are manually counted using an inverted microscope. After counting, the same cultures are then processed to measure the intracellular ATP concentration using the reagents included in the kit. Prior to processing, an ATP standard curve and high and low controls are performed. When the total colony count is plotted against the iATP concentrations, a direct correlation between the 2 parameters should be obtained, as shown in the diagram below.



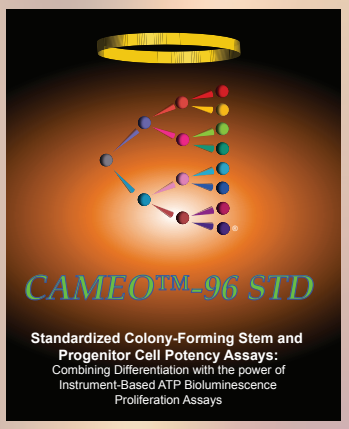
Since the ATP bioluminescence proliferation assay is calibrated and standardized against an external ATP standard curve with controls, performing the CFC assay under exactly the same conditions, means that the CFC assay has also been calibrated and standardized against the ATP assay.

Because of the correlation produced, total colony counts can be expressed directly as standardized iATP concentrations.

CAMEO-96 STD Assay Kits

CAMEO®-96 STD is available to standardize the CFC assay for multiple cell populations:

- CFC-GEMM alone
- HPP-SP and CFC-GEMM
- CFC-GEMM + BFU-E + GM-CFC and Mk-CFC
- HPP-SP, CFC-GEMM, BFU-E, GM-CFC, Mk-CFC, T-CFC, B-CFC and a background.



CAMEO®-96 STD

Standardizing the CFC Assay against HALO®

Combining Differentiation with the Power of Instrument-Based ATP Bioluminescence Technology

HemoGenix® provides a contract service to perform all quality control, stem cell potency and patient monitoring assays. Please contact HemoGenix® for more information and pricing.

For the HemoGenix® Assay Kit Catalog with all of the kits described in this brochure, please visit our website at www.hemogenix.com to download the complete catalog. For U.S. customers, online ordering of assay kits is also available.

HemoGenix(r) accepts purchase orders and most major credit cards for its assay kit products and accessories.

HemoGenix® offers individual and small group training at its facility in Colorado Springs. Alternatively, training can also be performed at your facility.

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