THE PROBLEM
Since 1971, when Pike and Robinson first modified the Colony Forming Cell (CFC) Assay to detect human cells, this assay has been used to measure stem cell product potency for stem cell transplantation and eventual stem cell storage. But the CFC assay was never designed and developed as a potency assay, but rather as an assay for investigational use. In accordance with the Code of Federal Regulations, article 21 CFR 610.10 states that “tests for potency shall consist of either an in vitro or in vivo test, or both, which have been specifically designed for each product so as to indicate its potency”. Now with improved technology, the CFC assay is not the assay of choice for four reasons:

1. Subjectivity: A potency assay should be based on an independent, instrument-based reading and not manual enumeration of colonies.

2. Calibration and Standardization: A potency assay should be calibrated against an external standard, thereby allowing the assay to be inter-calibrated and validated.

3. Assay Time: According to the FDA, “potency testing for living product cells may be compromised by extensive assay time”. The 14 day culture compromises results.

4. Assay: The CFC assay is a differentiation assay. Differentiation is the process whereby an undifferentiated cell, i.e., a stem cell (by definition), acquires the features of a specialized cell. Proliferation is defined as the expansion of cells by the continuous division of two identical daughter cells. Proliferation occurs prior to differentiation. Without proliferation, differentiation would not occur. And differentiation is a detailed program requiring prior proliferation. The CFC assay therefore measures differentiation potential. As such, not only was the CFC assay not designed to measure potency, but it provides indirect information. The CFC assay is the incorrect assay to use for stem cell potency measurements.

THE SOLUTION TO MEASURING STEM CELL POTENCY
These, and many other drawbacks of the CFC assay were taken into account in the development of the HALO™ Stem and Progenitor Cell - Quality Control (SPC-QC) Platform. The original HALOTM assay, the High Proliferative Potential - Stem and Progenitor Cell (HPP-SP) assay, is a novel 96-well plate, methylcellulose, clonogenic assay, which instead of counting colonies, directly measured cell proliferation of individual cell populations by virtue of the fact that, when stem and progenitor cells are stimulated with growth factors, an increase in intracellular ATP (ATP) concentration occurs that directly correlates with proliferation. By releasing ATP from the cells, i.e., as a light-emitting substrate for a luminometer, reaction to produce bioluminescence in the form of light detected in a plate luminometer. HALO™ is calibrated and therefore standardized using an ATP standard dose response curve prior to measuring the samples. This adds intra- and inter-laboratory consistency of results. It also allows various procedures which may sample potency, e.g., media preparation, cryo-preservation and thawing to name but a few, to be compared and standardized between laboratories.

Although a clonogenic assay has traditionally been considered the only way to detect lymphohematopoietic stem and progenitor cells, we now demonstrate that stem and progenitor cell populations by virtue of the fact that, when stem and progenitor cells are stimulated with growth factors, an increase in intracellular ATP (ATP) concentration occurs that directly correlates with proliferation. By releasing ATP from the cells, i.e., as a light-emitting substrate for a luminometer, reaction to produce bioluminescence in the form of light detected in a plate luminometer. HALO™ is calibrated and therefore standardized using an ATP standard dose response curve prior to measuring the samples. This adds intra- and inter-laboratory consistency of results. It also allows various procedures which may sample potency, e.g., media preparation, cryo-preservation and thawing to name but a few, to be compared and standardized between laboratories.

THE ASSAYS

HALO™-96 MeC SPC-QC PROTOCOL for a SINGLE STEM CELL POPULATION

Step 1. Prepare HALO™-96 MeC SPC-QC PROTOCOL for a SINGLE STEM CELL POPULATION

Step 2. Add suspension to each pre-dispensed Master Mix to tube so that the luminescence. Dispense 100µl into 4 replicate wells (yellow to red).

Step 3. Mix a positive displacement repeater using 3rd Generation Luminescence Reagent.

Step 4. Incubate 10 min.

Step 5. Count colonies in each well.

Step 6A. Add 100µl of luminescence monitoring reagent and mix.

The results from these new assays is that colony counts can now be expressed as standardized ATP equivalent concentrations.

STANDARDIZING THE COLONY-FORMING CELL ASSAY against HALO™-96 MeC to PRODUCE a DUEL PROLIFERATION / DIFFERENTIATION POTENCY ASSAY at 14 DAYS: CAMEO™-96 STD

The only procedure to standardize the traditional colony-forming cell assay is against another assay that is validated and can be calibrated and standardized. Since HALO™-96 MeC is calibrated against the colony-forming cell assay and the CFC assay is a calibration and standardized assay, the reverse process can standardize the colony-forming cell assay, against both assays performed under exactly the same conditions. No conditions or requirements are necessary other than the same conditions.