

## Asymmetrex's TORTOISE Test™ and RABBIT Count™ Platforms For Kinetic Stem Cell Counting

### What is kinetic stem cell (KSC) counting?

KSC counting is a method for determining what fraction of cells in isolated preparations of mammalian organ and tissue cells are stem cells. The method can be used to determine the *stem cell-specific fraction (SCF)* of organ and tissue cell preparations isolated from any vertebrate animal, including the SCF of important human tissues in cell research, medicine, and drug development.

### Why is knowing the SCF important?

In areas of cell research like cancer cell biology and tissue cell physiology, scientists have sought a means to quantify the number stem cells in their research experiments for decades with few solutions. The only methods currently available for counting tissue stem cells are tests for human blood stem cells based on their ability to establish the human blood cell system in mice with compromised immune systems. These assays are very expensive, requiring as many as 50 mice; and protracted, taking 12-16 weeks to obtain an estimate of the SCF of a single sample. It is no surprise that these reconstitution assays have proven too costly, difficult, and unreliable to be used routinely. A further limitation is that mouse reconstitution assays only work for blood stem cells.

The SCF is needed to define the stem cell-specific dosage for stem cell treatments and gene therapy trials as well, since many of the latter target blood stem cells. The two major approved stem cell therapies – transplantation of adult blood stem cells and transplantation of umbilical cord blood stem cells – would both benefit from a method to determine the stem cell-specific dose of treatments. Although adult blood stem cell treatments have a failure rate of a few percent that might be attributed to an insufficient dose, the rate for cord blood transplants approaches 20 percent. Currently, there is no way to predict which unit of cord blood has sufficient stem cells. Most patients treated with cord blood stem cells are children with leukemia. Advancing to knowing the stem cell-specific dose of cord blood units would greatly reduce the anguish and death that these children and their families face. There is also a need for stem cell-specific dosing in adult blood stem cell treatments, too. Although the treatments have a much higher success rate, donor stem cells are scarce. If the SCF of donor stem cells were known, in many cases there might be a sufficient dose for treating multiple patients, instead of only one as now.

There are many companies working to develop manufacturing processes for expanding therapeutic tissue stem cells and for harvesting products produced from them (e.g., microvesicles). Without a means to determine the SCF currently, this process engineering proceeds blindly. The ability to determine the SCF at any point in production, particularly in the starting tissue cell substrate, is likely to prove to be an effective predictor of downstream production quality.

The ability to monitor the SCF has an important application in pharmaceutical and biopharmaceutical drug development. Because of the essential role stem cells have in maintaining and renewing organs and tissues, drugs that are stem cell-toxic cause chronic organ failure. Induction of chronic organ failure (e.g., liver failure and bone marrow failure) appearing in Phase II and Phase III clinical trials is a major cause of drug development failures. Pharma companies attempt to identify these dreaded drug candidates in pre-clinical animal studies, but many still are not discovered until they injure patients in expensive later clinical trials. The ability to monitor SCF provides an inexpensive means to screen out such catastrophic drug candidates much earlier at much reduced cost. The same SCF tool can be used to discover beneficial drug candidates that cause stem cells to expand in number for biomanufacturing and potentially act as wound repair agents in the body.

### How does KSC counting work?

Asymmetrex's TORTOISE Test™ technology uses computer simulation to determine how many stem cells are present in a tissue cell culture by analyzing how fast the cells in the culture, produced by the division of

the stem cells, appear and disappear over time (*i.e.*, kinetics). The TORTOISE software uses machine learning principles and runs in a secure cloud computing architecture.

**What is the input data needed for the TORTOISE software?**

The input data for the TORTOISE software are simple total viable cell count data from serially passaged cell cultures. Any cell culture format that allows total cell number determination can be used, including adherent, suspension, and microcarrier.

**What information does the TORTOISE software provide the user?**

**Stem cell-specific properties**

1. **SCF** of the starting sample and the SCF at any time during its culture
2. Stem cell-specific death rate at any time during the culture
3. Average cell cycle time of stem cells
4. Relative frequency of stem cell divisions that produce two stem cells (*i.e.*, symmetric divisions) *versus* stem cell divisions that produce committed tissue cells (*i.e.*, asymmetric divisions)

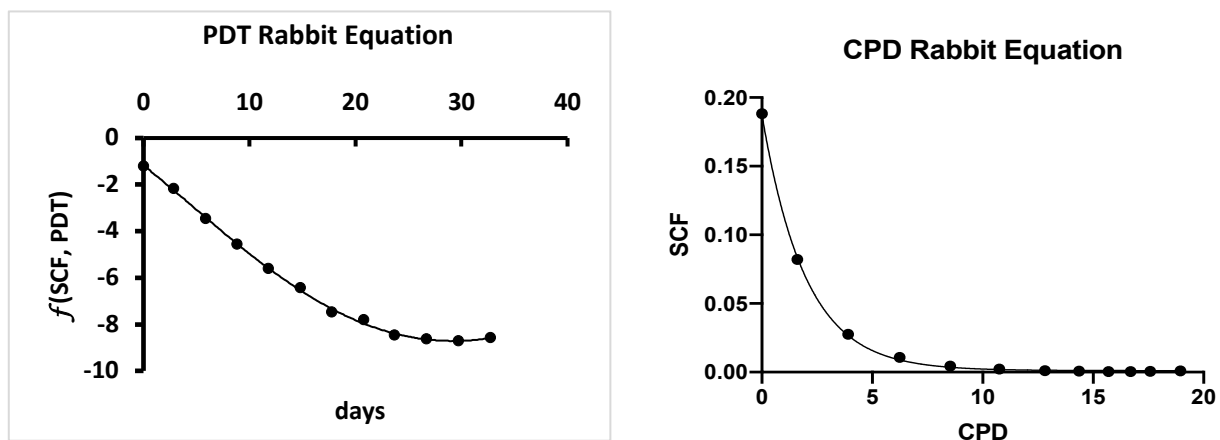
**Committed cell-specific properties**

1. Proliferating committed cell fraction and terminally-arrested committed cell fraction of the starting sample and at any time during its culture
2. Specific death rate at any time during the culture
3. Average cell cycle time for proliferating committed cell fraction

**What is a RABBIT Count?**

The computational outputs from the TORTOISE software were used to discover mathematical equations (“*rabbits*”) that relate the SCF for cultured tissue cells to two widely used parameters for quantifying cell proliferation rates. The two parameters are the related factors population doubling time (**PDT**) and cumulative population doublings (**CPD**). PDT is the amount of time it takes for the total cell number of a culture to double. CPD, is a running summation of the number of times a culture’s total cell number has doubled. Both PDT and CPD are commonly determined and reported values in tissue cell research.

Fig. 1. below shows the graphical out for a PDT rabbit equation and its associated CPD rabbit equation developed for CD34<sup>+</sup>-selected human cord blood cells (*i.e.*, blood hematopoietic stem cells).



**Figure 1. Rabbit equations for rapid determination of the SCF of human cord blood (CD34<sup>+</sup>-selected source).**

**What can a user do with RABBIT equations?**

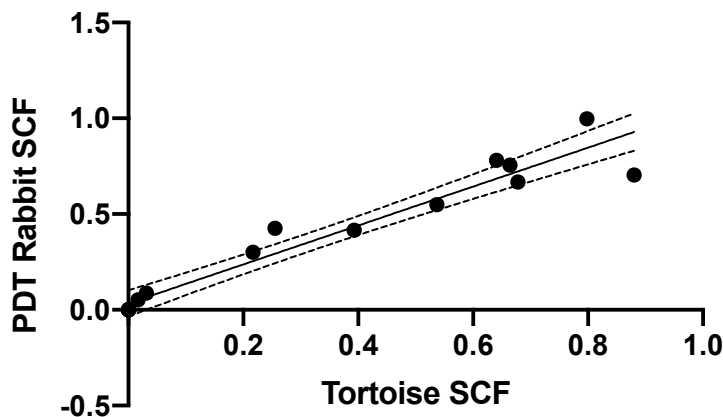
With a PDT rabbit equation, if the user enters the PDT for a cultured tissue cell preparation and the number of days of serial culture, the SCF can be calculated directly. A PDT determination only requires two total cell number counts for a culture at two different points in time (e.g., at 0 hours of culture and at 72 hours of culture).

With a CPD rabbit equation, if the user knows the initial SCF for a tissue cell preparation (e.g., determined with a PDT rabbit equation), the SCF of the preparation can be predicted with a high degree of confidence for any future number of CPDs of the preparation.

**Has KSC counting been validated?**

The validation of the ability of the TORTOISE software to determine the SCF of tissue stem cells from several different therapeutically important human tissues has been published in both patents and a recent peer-reviewed scientific report (1-4). Validation strategies have included comparison to human blood system reconstitution in mice; fractionation analyses with antibodies that enrich for tissue stem cells; analyses with both stem cell-toxic and stem cell-activating agents; and comparisons to independent indicators of the specialized asymmetric tissue cell renewal kinetics of tissue stem cells.

The ability of Rabbit equations to accurately determine SCF is shown in Fig. 2 below. PDT rabbit equations derived for human blood (hematopoietic) stem cells (HSCs from bone marrow and cord blood), human liver, and human fat tissue-derived mesenchymal stem cells (MSCs) have been evaluated. Tissue cell preparations were also cultured with different supplemented growth factors and stem cell-active agents.



**Figure 2. Correlation analysis between rapid determination of SCF with PDT rabbit equations and determination of SCF by TORTOISE software analyses of serial cell culture data.** Slope of regression line = 1.02 (95% CI, 0.87-1.17; *n* = 16 independent rabbit equations). Dotted lines indicate 95% confidence boundaries.

**Additional questions?**

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**References**

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Web link

<https://asymmetrex.com/wp-content/uploads/2021/04/KSCcount.pdf>