

## The AlphaSTEM Test Contract Service

**General:** The AlphaSTEM Test Contract Service provides cell kinetics data that are specific for adult tissue stem cells, non-stem cycling cells (e.g., committed progenitor cells, transiently amplifying cells), and terminally-arrested cells (e.g., non-dividing differentiated cells) that make up complex tissue cell preparations in culture.

**Basic Determinations:** Table 1 outlines the tissue cell type-specific cell kinetics parameters provided by the AlphaSTEM Test Contract Service.

**Table 1. Cell Kinetics Parameter Determinations**

<u>Tissue Stem Cells</u>	<u>Non-Stem Cycling Cells</u>	<u>Terminally-Arrested</u>
Number vs. time	Number vs. time	Number vs. time
Death rate vs. time	Death rate vs. time	Death rate vs. time
Cell cycle time for symmetric s-r*	Cell cycle time	
Cell cycle time for asymmetric s-r		
Symmetric self-renewal rate		
Asymmetric self-renewal rate		
SC unit generation number**		
<u>Density-dependence of symmetric s-r rate</u>		
*s-r, self-renewal; **number of cell generations between stem cell and terminally differentiated cells produced in culture		

**Test Article Evaluations:** The AlphaSTEM Test can be used to quantify the effects of supplemented test articles (e.g., growth factors, culture medium additives, drug candidates, environmental toxicants) on any of the cell kinetics parameters listed in Table 1 above.

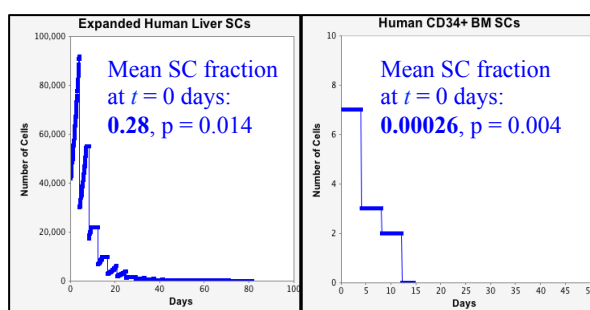
**Requirements:** AlphaSTEM Test analyses require the input of viable total cell count data from replicate (triplicate minimum) tissue cell cultures serially passaged by one of the following schedule options:

- I. Addressed serial cultures passaged at a constant split fraction after attaining a constant degree of confluency.
- II. Addressed serial cultures passaged at a constant cell number after culture for a constant time interval.
- III. Addressed serial cultures passaged at a constant split fraction after a constant time interval.

Culture series must be continued until two consecutive passages occur with no increase in cell number over the input cell number. Asymmetrex will conduct the required cell culture studies, or they can be conducted by the client with an appropriate reduction in the cost for subsequent cell kinetics determinations.

**Cost:** Negotiated based on project features.

**EXAMPLES:**



**Fig. 1.** Examples of AlphaSTEM Test stem cell counting results from serial passage (Option III) of expanded human liver stem cells (300,000 total cell input) and human CD34+ bone marrow stem cells (47,000 total cell input). Note detection of symmetric self-renewal by liver stem cells, but not hematopoietic stem cells, between passages (vertical lines).

**Table 2. Comparison of AlphaSTEM Cell Kinetics Parameters for Different Tissue SCs and Conditions**

<u>Cell Kinetics Parameter</u>	<u>Expanded Liver</u>	<u>CD34+ BM</u>	<u>CD34+ BM/BCNU*</u>
<b>Stem Cells</b>			
Initial Fraction	0.28 (0.014)	0.00026 (0.004)	<b>0.00013 (0.0009)</b>
Death Rate	0.042 (0.041)	0.0014 (NS)	0.0 (NS)
Symmetric Cell Cycle Time	30h (0.0002)	7.8h (< 0.0001)	8.2h (NS)
Asymmetric Cell Cycle Time	16h (0.0001)	7.0h (0.0002)	7.6h (NS)
Symmetric self-renewal rate	0.24 (0.048)	0.0013 (NS)	0.0 (NS)
SC unit generation number	15 (0.0001)	13 (< 0.0001)	14 (NS)
<b>Non-stem cycling cells</b>			
Death Rate	0.009 (NS)	0.02 (< 0.0001)	<b>0.01 (&lt; 0.0001)</b>
Cell Cycle Time	18h (0.0003)	6.8h (< 0.0001)	6.4h (NS)

\*BCNU = 50 microM BCNU, a hematopoietic stem cell toxin; values are mean (p); NS, not statistically significant at the p = 0.05 level. BCNU p values refer to comparisons with the control condition.