

One Cell Systems, Inc.
Screening Service
Gel Microdrop Growth Assay

The Cell Screening Service is comprised of five phases: 1) Evaluation of the Cell Line after encapsulation; 2) Assessment of Reagents; 3) Assay Optimization; 4) Cell Screening and Selection; and 5) Delivery of Sorted Cells. One Cell Systems works closely with each client to address specific goals and requirements, and to isolate cells of interest in a timely, cost-effective manner.

Length of Study

Approximately 6-10 weeks; the study includes the following: assay development and optimization, screening, selection and propagation of cells. The major factors influencing assay development are: 1) the quality of the reagents for discriminating cells and 2) the growth kinetics of the cell line.

Reagents Supplied by Client

Cell Line

Cryopreserved cells should be delivered to One Cell Systems on dry ice along with procedures for initiating cultures, sub-culture and cryopreservation. If a sub-cloned cell line will be screened, an aliquot of the parental cell line should also be sent.

Reagents

Reagents for discriminating cells should be provided by the customer. Antibodies for surface staining should be affinity purified.

Approved preferential sera, proprietary cell culture media and other reagents specific to the client's system may also be required.

Cell Assessment and Assay Development

Evaluation of the Cell Line

The viability and growth of cell line of interest after encapsulation will be evaluated. Compatibility of the cells with surfactants used in the Growth Assay, recovery of viable cells from GMDs, and culture methods will also be tested and optimized. The culture will be expanded, aliquoted and frozen.

Assessment of the Reagents

The appropriate concentrations of each reagent will be determined empirically to further optimize the specific GMD Growth Assay. Evaluation of alternative reagents can be performed at additional cost.

Assay Optimization

In this phase, optimal conditions will be established for: 1) assaying viable, encapsulated cells, and 2) performing flow sorting of the cell line. The assay conditions established during the Assay Development Phase will be used to prepare a pilot GMD Growth Assay. The assay parameters that result in a strong signal will be determined and validated. To establish good resolution, the incubation time, during which there is a clear shift above background, will be determined.

Cell Screening and Selection

Growth Assay and Sorting

Using the conditions established in the assay development and optimization phases, a large sample will be prepared by encapsulating 3 - 4 aliquots of 1×10^6 cells. The samples will be pooled and the Growth Assay performed. The cells will be sorted using a stringent gate for fluorescence or side scatter. Cells of interest will be selected by sorting. Additional cells may be isolated from other Growth Assays of the same cell line.

Culture and Screening of the Cells

The selected cells will be cultured for up to three weeks at which point they will be assayed for viability.

Cryopreservation and Delivery of Sorted Cells

Frozen aliquots of the sorted cells of interest will be delivered at the end of the study. Procedures for cryopreservation should be provided in advance. Alternatively, the sorted cells may be stabilized in culture for 24 – 48 hours, after which the flask will be filled with media and the cells shipped at ambient temperature.

**Growth Assay
Cell Screening Program
Service Agreement**



Service Agreement/Material Transfer



Evaluate Cell Line

Evaluate cell viability and growth in Gel Microdrops
Recover viable cells from Gel Microdrops
Surfactant compatibility
Culture and processing methods



Assess Reagents

Evaluate reagents
for efficacy in Growth Assay
Generate kinetics growth curve



Optimize Assay

Establish optimal conditions for assessing viable, encapsulated cells
and flow sorting of the specific cell line



Screen and Select Cell

Perform Growth Assay
Select cells of interest
Culture
Assay for viability



Cryopreserve and Deliver

Growth Assay Cell Screening Service

Stage	Time	Payment Due
Service Agreement/Material Transfer		\$4,600 in advance
Evaluate Cell Line	2-4 weeks	\$4,600 at report
Evaluation of cell viability and growth in Gel Microdrops Recovery of viable cells from Gel Microdrops Surfactant compatibility Culture and processing methods		
Assess Reagents	1-2 weeks	\$4,600 at report
Evaluation of labelling reagents		<i>Evaluation of alternative antibodies can be performed at additional cost.</i>
Optimize Assay	1-2 weeks	\$4,600 at report
Establish optimal assay conditions and performing flow sorting		
Screen and Select Cells	2-4 weeks	\$4,600 at report
Select Cells of Interest Culture Re-Assay		<i>Additional rounds of enrichment can be performed at additional cost.</i>
Cryopreserve and Deliver Cells frozen and delivered Final report and recommendations	1 week	

Client has the option to continue the program at each stage.
 OCS will continue only upon receipt of written notice to proceed.
 Client also has the option of performing additional studies at each stage, i.e.: to evaluate alternative antibodies, cell lines, etc., at the noted price.