

## **Secretion Assay Cell Screening Service Gel Microdrop**

The Cell Screening Service is comprised of five phases: 1) Evaluation of the Cell Line after encapsulation; 2) Assessment of Reagents; 3) Secretion Assay Optimization; 4) Cell Screening and Selection; and 5) Delivery of the Sorted Cells. One Cell Systems works closely with each client to address specific goals and requirements, and to isolate high secreting cells 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, and screening of protein production by supernatant assay. The major factors influencing assay development are 1) the quality of the antibody pair used for capture and detection and 2) the kinetics of protein secretion by each particular 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.

#### *Antibody Pair*

A validated, non-overlapping antibody pair directed against the target protein is required. Antibody pairs used in a standard sandwich immunoassay sensitive to 10 pg/mL are usually effective in the GMD Secretion Assay. Antibodies should be affinity purified.

The biotinylated antibody will be used as the capture antibody, which will be attached to the CelBioGel matrix through a streptavidin bridge. The other fluorochrome-labeled antibody will be used to detect the captured secreted protein in GMDs. R-phycoerythrin (R-PE) or fluorescein isothiocyanate (FITC) are recommended for optimal detection using flow cytometry.

#### *Protein Sample*

Approximately 1.0 mg of purified protein is necessary to generate assay standards and positive controls. Alternatively, an aliquot of the cell culture supernatant with a known concentration of secreted protein may suffice.

*Approved preferential sera, proprietary (biotin-free) 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 Secretion 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 antibody pair, protein sample and other reagents will be tested for efficacy in the GMD Secretion Assay. The target protein sample, biotinylated capture and fluorochrome-labeled detection antibodies will be used with unoccupied GMDs to generate a signal titration curve for different protein concentrations. The appropriate concentrations of each reagent will be determined empirically to further optimize the specific GMD Secretion Assay. Evaluation of alternative antibodies can be performed at additional cost.

## **Assay Optimization**

In this phase, optimal conditions will be established for 1) assaying protein secretion from 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 pilot GMD Secretion Assays. The assay parameters that result in a strong signal from secreted protein without saturating GMDs will be determined and validated. To establish good resolution between high and low secretors, the incubation time, during which protein produced in the GMDs gives a clear shift above background, will be determined.

## **Cell Screening and Selection**

### *Secretion 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  $1 \times 10^6$  cells. The samples will be pooled and the Secretion Assay performed. The cells will be sorted using a stringent gate for fluorescence. High secretors will be selected by sorting those cells exhibiting the highest fluorescence, approximately 0.5% – 0.1% of the secreting cell population (at least 1,000 – 2,500 cells). A typical CHO cell line may exhibit a cloning efficiency of 40%; thus the 1,000 – 2,500 cells collected during the Sorting Phase will result in 400 – 1,000 cells available for further processing. Additional cells may be isolated from other Secretion 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 protein production. Supernatants will be harvested and screened by Gel Microdrop Supernatant Assay.

### **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 ambient at temperature.

**Secretion Assay  
Cell Screening Program**



**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 Antibody Pair, Target Protein and other reagents  
for efficacy in Secretion Assay  
Generate titration curve



**Optimize Assay**

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



**Screen and Select Cell**

Perform Secretion Assay  
Select high secretors  
Culture  
Assay for protein production



**Cryopreserve and Deliver**

## Secretion Assay Cell Screening Service

Stage	Time	Payment Due
Service Agreement/Material Transfer		\$6,000 in advance
Evaluate Cell Line	2-4 weeks	\$6,000 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	\$6,000 at report
Evaluation of antibody pair, target protein and other reagents		<i>Evaluation of alternative antibodies can be performed at additional cost.</i>
Optimize Assay	1-2 weeks	\$6,000 at report
Establish optimal conditions for assessing protein secretion and performing flow sorting		
Screen and Select Cells	2-4 weeks	\$6,000 at report
Select High Secretors Culture Assay for protein production		<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.