

Bulk provision of receptor membranes for high-throughput screening

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Bulk provision of receptor membranes for high-throughput screening

Key words: Cell Factory • SPA Scintillation Beads • assay development

The recent growth in the use of cell lines for drug discovery, particularly for high-throughput screening (HTS), has posed a number of challenges including maintaining batch performance, control of cell production scheduling, and capability and capacity management. In response to the increasing demands for cells and assay components derived from cells (e.g. microsomal membrane fractions), the Cell Factory Service provides an effective solution.

Cell Factory uses a partnership approach to delivering customized solutions that increase the capability and capacity of researchers using either whole cells or cell-derived components. To complement traditional approaches to cell culture, we have optimized the use of microcarrier technology for the scaled production of cells. When used in combination with stirred tank bioreactors, this system provides a reproducible format for bulk cell production. The combination of microcarriers and bioreactors allows up to 3×10^{10} cells to be grown as a single batch. Cells cultured by this method have been validated in assays and shown to meet performance criteria required for use in HTS.

Here we describe a process for the large-scale production of microsomal membrane fragments from a recombinant CHO cell line expressing the muscarinic-M1 receptor. The protocol has been developed to provide maximum yields of membrane from harvested cells. The yield is calculated by protein determination and the quality of the membrane fractions is assessed by a receptor binding Scintillation Proximity Assay (SPA).

Materials

Products used

[³ H]QNB, 250 µCi	TRK604-250UCI
Cytodex™ 3 microcarriers	17-0485-01
WGA PVT SPA Scintillation Beads	RPNQ0001

Other materials required

CHO-M1 cell line	In-house
Spinner flask or stirred tank bioreactor	
Trypsin/EDTA solution	(Sigma) T3924
BCA Protein Assay Kit	(Pierce) 23225
CellSTACK™ Culture Chamber	(Corning) 3313

Methods

Preparation of cell pellet

CHO-M1 cells were grown to ~ 80 to 95% confluence on Cytodex 3 microcarriers or 5-layer CellSTACK Culture Chambers. Cells maintained on Cytodex 3 microcarriers were cultured in both spinner flasks and stirred tank bioreactors. Trypsin/EDTA was used to detach cells from all surfaces, followed by harvesting by centrifugation. The cell pellets were stored overnight at -70°C.



Preparation of membrane fractions

Microsomal membrane fractions were prepared using an in-house protocol developed for handling large-scale cell cultures. Briefly, the cell pellet was thawed on ice then subjected to mechanical homogenization followed by differential centrifugation to obtain microsomal membrane fractions. Protein concentration was determined prior to storage at -70°C using a BCA Protein Assay Kit.

Table 1. Cell and membrane yields from cultures

	Cell yield	Total membrane protein yield (mg)
CellSTACK culture	6.7×10^9	181
Spinner culture	2.9×10^9	46.5
Bioreactor culture	2.8×10^9	77.4

SPA receptor ligand binding assay

Muscarinic-M1 receptor microsomal membrane fractions from a CHO-M1 stable cell line were incubated with [³H]QNB together with 300 µg or 1000 µg of Wheat Germ Agglutinin (WGA) PVT SPA Scintillation Beads. Following addition of the individual assay components to the appropriate wells of 96- and 384-well microplates, all plates were stored overnight at room temperature in the dark and then counted on a Wallac™ Microbeta™ Scintillation Counter (1 min/well, 5 to 360 window setting).

Table 2. Assay protocol for 96-well microplate

	Totals (µl)	NSB (µl)	Background (µl)
Assay buffer	50	-	100
[³ H]QNB	50	50	50
Membrane (2.5 µg)	50	50	-
Atropine (1 µM)	-	50	-
SPA Scintillation Bead (1000 µg)	50	50	50

Table 3. Assay protocol for 384-well microplate

	Totals (µl)	NSB (µl)	Background (µl)
Assay buffer	15	-	30
[³ H]QNB	15	15	15
Membrane (2.5 µg)	15	15	-
Atropine (1 µM)	-	15	-
SPA Scintillation Bead (300 µg)	15	15	15

Results

Optimization of membrane for the SPA

Figure 1 compares the performance of membrane fractions derived from each of the indicated culture formats. At typical assay amounts (1.25 to 5 µg of membrane/well), all three methodologies provide membrane fractions that produce comparable signals in the SPA. The optimum amount of membrane per well is 2.5 µg for both 96- and 384-well formats based on the signal:background ratio. The results show that growth of the CHO-M1 cell line on microcarriers does not impair the performance of the membrane fraction in the SPA when compared to cells cultured as monolayers in CellSTACK Culture Chambers.

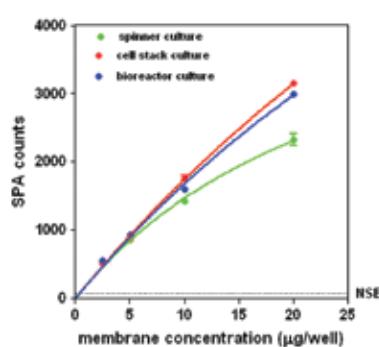


Fig 1. Comparison of microsomal membrane fractions prepared from the CHO-M1 cell line grown under differing culture conditions. Increasing amounts of membrane were added to a fixed mass of WGA PVT SPA Scintillation Beads. Results are means \pm SEM (N = 3).

Z' analysis for 96- and 384-well assay formats

For biochemical assays, Z' values between 0.5 and 1 are considered appropriate for HTS. 96- and 384-well assays were set up as described. The data show that assays using membrane fractions derived from microcarrier cultures generated a Z' value of 0.82 in a 96-well assay (Fig 2A) and 0.75 in a 384-well assay (Fig 2B). These values are indicative of assays suitable for use in HTS.

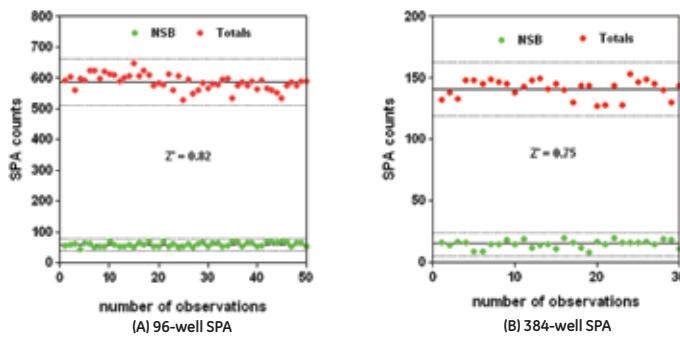


Fig 2. Z' analysis conducted with membranes derived from the bioreactor culture of CHO-M1 cells in (A) 96-well and (B) 384-well assays.

Competition binding

Figures 3A and 3B and Table 4 compare the different membrane preparations and assay formats for atropine inhibition of [³H]QNB binding to the M1 receptor. Comparable IC₅₀ values were obtained for all membrane preparations in both assay plate formats. The data confirm that the pharmacology of M1 receptor membranes prepared from CHO-M1 cells grown on microcarriers is comparable to membranes prepared from conventional tissue culture approaches.

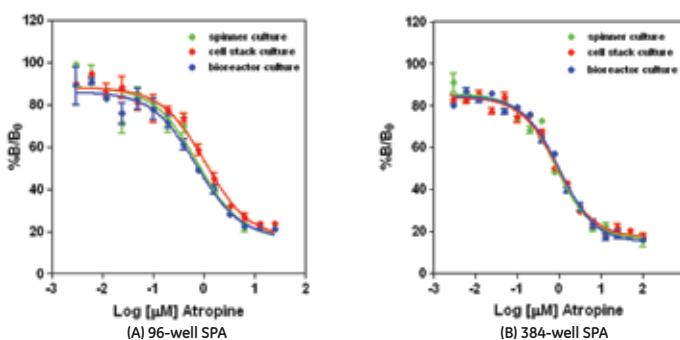


Fig 3. CHO-M1 cell membranes were incubated with increasing concentrations of atropine. Assays were performed in (A) 96-well and (B) 384-well assays. Results are means \pm SEM (N = 3).

Table 4. IC₅₀ values for atropine antagonism of [³H]QNB binding to the muscarinic-M1 receptor

	IC ₅₀ (nM) 96-well assay*	IC ₅₀ (nM) 384-well assay*
CellSTACK culture	1066	902.8
Spinner flask culture	751.2	825.6
Bioreactor culture	701.5	1001

* Values derived from Figure 3.

Stability of CHO-M1 membrane fractions

Figure 4 illustrates the stability of CHO-M1 membranes over a 20 week period. At each time point, increasing amounts of membrane were added to the 96-well SPA and the signal measured. The membrane preparation showed an acceptable level of stability, in particular at the amounts used in a typical SPA receptor ligand binding assay (i.e., 1.25 to 5 μ g).

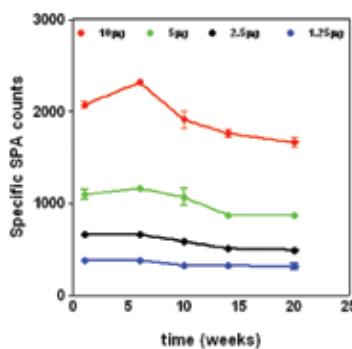


Fig 4. Stability of CHO-M1 membranes prepared from the bioreactor culture. Results are means \pm SEM (N = 3).

Conclusion

The data show that microcarrier based cultures produce membrane fractions that are of equivalent quality to those produced by standard cell culture methods. The scalability of both microcarrier based culture systems and the membrane production process allows the preparation of large quantities of membranes that meet HTS performance criteria.

Cell Factory offers a convenient solution for the large-scale production of cells and cell-derived components. The combination of microcarrier based approaches with environmentally controlled bioreactors provides a system that ensures recombinant cell lines are maintained under optimal growth conditions. The ability to combine large-scale cell production and processing of the cells as a single batch avoids potential batch-to-batch variation in performance.

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