Apparatus for High-Throughput Filtration of Cell Lysates

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Introduction

The clarification of cell requires separation of cell debris, colloids, insoluble precipitates, aggregates and other contaminants before the target molecule is isolated and characterized. Centrifugation is commonly used to remove large contaminants as much as possible to clear a lysate solution. Unfortunately centrifugation is not readily adaptable to automation for high-throughput applications. Here, we present a performance study of an apparatus for crude lysate filtration comprising multi-well filtration plate to clear cell lysates. Each well comprises an upper filtration zone; a lower filtration zone; a conical flow director zone; and, an elution tip, wherein the upper filtration zone and the lower filtration zone are separated by a retainer ring disposed within the lower filtration zone. The upper filtration zone comprises an upper collection zone, a depth filter media zone, and a deep bed filtration zone; and, the lower filtration zone comprises the retainer ring, a supported membrane and a lower bed filtration media.

Methods

Preparation *of E. coli* cells pellet: An *E.coli* pET-based vector was used for expression of a sea coral protein. The plasmid was transformed into BL21 DE3 and was grown for 2 days at 17 degrees C in auto-induction media in 1ml cultures in a 96-well, deep-well block. Pellets were collected by spinning at 4000 x *g* for 10min.

Preparation of insect cells pellet: P3 virus for a sea coral protein was used to infect 2×10^6 Sf9 cells in ESF921 media. Cells were grown in 1ml cultures in a 96-well, deep-well block at 27 degrees C for 72hrs. Pellets were collected by spinning at 4000 x *g* for 10min.

Cell lysis procedure: Cells pellet plate and required total volume of lysis buffers (B-PER, Bacterial Protein Extraction Reagent and I-PER, Insect Protein Extraction reagent, Thermo Scientific, Rockford, IL) were thawed to room temperature. 1.0 mL of lysis buffer was then added to each of the 48 randomly selected cell pellet wells. The suspension was pipetted up and down to homogenize and the plate was incubated for 15 min at room temperature with gentle shaking on a plate shaker.

Filtration and centrifugation: Contents of 10 randomly selected pellet wells were fully transferred to either wells of the filter plate (Orochem Technologies Inc. Naperville, IL) or into centrifuge tubes (*Figure 1.*). The filter plate was placed on the collection plate and subjected for centrifugation at 1000 *x g* for 2 min in a C4.22 Jouan centrifuge equipped with plate adapter. Raw lysate transferred to centrifuge tubes were subjected for centrifugation at 8000 *g* for 5 min.

Sample volume recovery : Liquid from 10 randomly selected wells on a collection plate was fully transferred to previously weighed empty culture tubes. Tubes were weighed again with liquid and the difference in weight was used to verify reproducibility in sample volume recovery. Similar procedure was followed for supernatant from centrifuge tubes. Sample volume recovery was calculated as ratio of the two averages.

Total protein recovery : Total protein the samples was estimated using a commercial BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Total protein recovery was calculated as ratio of total protein content in centrifugation supernatant and filtrate.

Results

	1	2	3	4	5	6	7	8	9	10	11	12
A		f1	f2	f3	f4		f5	f6		f7		f8
B	f9	c1				f10		c2		f11	f12	
C		f13	f14		f15				f16	f17		f18
D	f19		f20	f21	c3	f22	f23	f24			f25	c4
E	c5	f26			f27			c6				f28
F						f29	f30		f31		f32	f33
G	f34	f35	f36	f37	c7		f38	c8				c9
H	f39	c10	f40		f41	f42	f43	f44	f45	f46	f47	f48

F	Filtration (F)
C	Centrifugation (C)
F	F – For sample volume recovery and Total protein recovery

Figure 1. Random selection of wells in cells pellet plate and selections for filtration and centrifugation.

Sample Volume Recovery:

Sample volume recovery showed excellent reproducibility for filtration plate, with recoveries around 90% for both cell lysates.

Sample Volume Recovery By Centrifugation

(n=10)	Average liquid weight (g)	STDEV	CV (%)
Bacterial lysate	0.8266	0.03856	4.7
Insect lysate	0.9431	0.02500	2.7

Sample Volume Recovery By Filtration

(n=10)	Average liquid weight (g)	STDEV	CV (%)	*Sample volume recovery (%)*
Bacterial lysate	0.7524	0.01332	1.8	*91.0*
Insect lysate	0.8426	0.01168	1.4	*89.3*

Total Protein Recovery:

Total protein recovery for bacterial lysate was slightly higher compared to insect lysate. In both cases, recoveries were good approaching and exceeding 80%.

Experiment	Total protein (mg/mL)		*Total protein recovery (%)*
	Filtrate	Centrifugation supernatant	
Bacterial lysate	4.85	5.48	*88.4*
Insect lysate	1.09	1.38	*79*

Discussion and Conclusions

We have demonstrated excellent performance of filter plate for application of lysate clarification. In cases of both lysates (bacterial and insect) sample volume recovery was highly reproducible while recoveries were ~90% as compared to centrifugation. Total protein recovery was also good ranging around 80% for insect lysate and 90% for bacterial lysate. Differences observed are possibly due to large difference in total cell content between the two types of cell pellets. The advantages of filtration protocol are low liquid retention, low adsorption of protein and results comparable or superior to high speed centrifugation. Orochem's lysate filter plate effectively removes cellular debris from cell lysates prior to purification of bio-molecules. This filter plate is a great tool that provides good bio-molecule recovery for high-throughput processing.

Acknowledgements: We thank Genentech, Inc. San Francisco, CA for the generous gift of cell pellets.