

Concentration of Soluble and Membrane Proteins with the Vivacell 100 Centrifugal Ultrafiltration Device

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Introduction

Protein Estimation

One of the most crucial steps at every stage of the protein purification protocol is concentration of the sample. This step enables the gathering of important data including specific activity, yield, recovery and the degree of/ or fold purification.

Protein samples can be concentrated using centrifugal ultrafiltration devices with membranes that have a specific molecular weight cut-off. For small volumes of protein solutions ranging from 0.1-10 ml there are many devices available on the market produced by a range of

manufacturers. Similarly, it is possible to concentrate large volumes ranging from 1-100 litres since the devices are available and these include the Sartocoon Slice (Sartorius) which consists of a membrane sandwiched between plastic supports.

The extract is pumped across the membrane under pressure and salts and molecules of lower mass than the size of the membrane pores permeate through the membrane, whilst higher mass molecules are retained.

However, if the extract to be concentrated is less than 1 litre there is a problem since this falls between the range of the low and high volumes devices available. In this

study, we have tested the Vivacell 100, an ultrafiltration device which fills this gap in concentration devices. The Vivacell 100 allows the concentration of 100 ml sample at any one time and uses gas pressure to force the samples against the membrane which is housed in a plastic support structure. However, the Vivacell 100 can also be used in the centrifuge.

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Axonal Growth Inhibition in the CNS of Higher Vertebrates

The axons of neurons in the mammalian CNS, including the human brain and spinal cord, fail to regrow following injury. Recent evidence from a variety of studies point towards axon-growth inhibitory factors in the environment of damaged axons as being responsible for the lack of axonal regeneration. In our laboratory, we have purified an axonal growth inhibitory protein that induces the collapse of axonal growth cones in culture (Figure 1). We have used this protein, as an example of a membrane protein to test the performance of the Vivacell 100 in the presence of detergent.

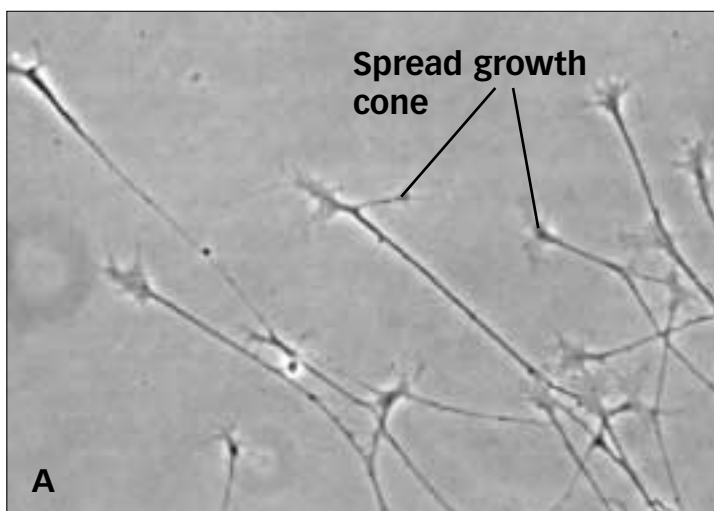


Fig. 1a: Neuronal outgrowth from an explanted dorsal root ganglion showing axons with normal or spread growth cones.

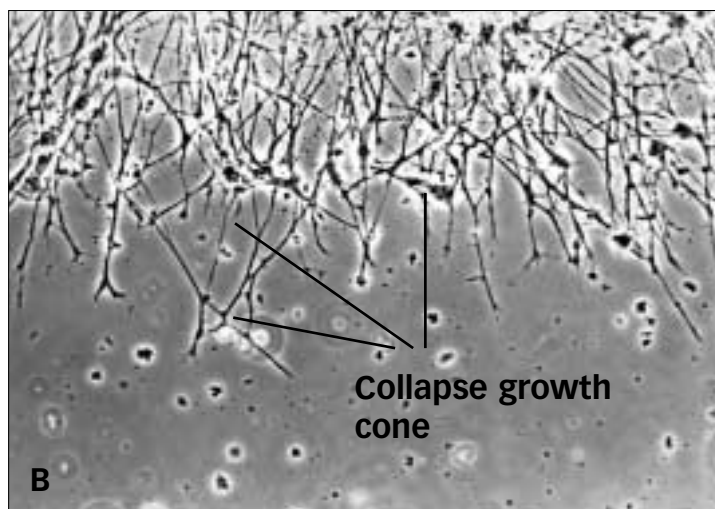


Fig. 1b: Neuronal outgrowth from an explanted dorsal root ganglion showing axons with collapsed growth cones following addition of 100 mls of an extract of embryonic membrane protein.

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Axon Guidance in the Vertebrate Retina

We have purified a diffusible protein that induces growth cone collapse of retinal ganglion cell growth cones and inhibits axonal outgrowth in collagen gel culture. We have used this soluble protein to test the performance of the Vivacell 100.

Materials and Methods

Collagen Gel Culture

400 μ l of Rat tail collagen 1 (Sigma) was mixed with 40 μ l of 10x Dulbecco's Minimal Essential Medium (DMEM) and 25 μ l of 7.5% sodium bicarbonate and vortexed for 1 min. A small drop of the collagen was pipetted into the well of a 4-well Nunc tissue culture plate. A small, 600 μ m², retinal explant and one embryonic day 6 chick lens were placed together on the drop of collagen separated by a distance of 8 mm. Another drop of collagen mixture was pipetted on to the tissues so that they were completely enclosed within collagen. The collagen was allowed to polymerise by placing the culture plate in a CO₂ incubator for 15 minutes at 37 °C. The culture wells were then filled with growth medium (3% Chick embryo extract in 1x DMEM).

Growth Cone Collapse Assay

Growing axons produce hand like structures at their tips known as growth cones. The addition of inhibitory proteins to cultures of dorsal root ganglion or retinal neurons induces a change in the morphology of growth cones from a spread structure to a collapsed stump. Thus, the growth cone collapse assay can be used to detect the presence of these proteins and quantify the amount of inhibition present in a sample. Membrane proteins need to be purified in the presence of detergents in order to keep them in solution. However, detergents need to be removed before their activity can be tested on growing neurons. This can be achieved either by dialysis, or by ultrafiltration or with the use of Vivapure spin columns. In order to mimic the natural environment of membrane proteins it is useful to incorporate them in to lipid liposomes. Liposomes are obtained by mixing the proteins with a combination of phospholipids (phosphatidylcholine and phosphatidylserine) and dialysing them against PBS. The liposome mixture is then added to cultures of growing neurons which are incubated for one hour and then fixed in 4% paraformaldehyde in PBS, pH 7.4. Growth cone collapse activity is expressed as the percentage of the total number of axons with collapsed growth cones.

Protein Estimation

The protein content of the filtrate and the retentate was measured using the Advanced Protein Assay.

Ultrafiltration

For the concentration of both the adult rat inhibitory membrane and the soluble embryonic lens protein in the Vivacell 100, a membrane with a 100 kDa molecular weight cut-off was used. In separate experiments, 100 ml of adult rat membrane protein and soluble embryonic lens protein was poured into the upper chamber of the Vivacell 100. To apply pressure across the membrane we used nitrogen gas at 4 bars. To keep the active proteins viable, the Vivacell 100 was packed on ice.

Results

Growth Cone Collapse

After concentration of the adult rat brain membrane and soluble embryonic lens proteins the growth cone collapse activity remains in the retentate of the Vivacell 100 (Table 1). Both the adult rat brain membrane protein and the soluble embryonic lens protein were concentrated 10 fold in the Vivacell 100. The protein concentrations before and after ultrafiltration are shown in Table 2.

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Table 1.

Sample	Filtrate		Retentate	
	Percentage of total number of spread growth cones	Percentage of total number of collapsed growth cones	Percentage of total number of spread growth cones	Percentage of total number of collapsed growth cones
Adult rat brain inhibitory membrane protein	99	1	3	97
Soluble embryonic lens protein	100	0	2	98
Phosphate buffered saline	98	2	NA	NA
Liposomes only	99	1	NA	NA

NA= Not applicable

Table 2.

Sample	Protein concentration at start of experiment (mg/ml)	Protein concentration mg/ml (Vivacell 100 Retentate)	Protein concentration mg/ml (Vivacell 100 Filtrate)	% Protein recovered
Adult rat brain membrane protein	4.6	45.1	0.5	98
Soluble embryonic lens protein	3.4	33.7	0.7	99

Conclusions

The excellent performance of the Vivacell 100 is reflected in the recovery of active protein. In this respect most of the active protein is recovered in the retentate with very little loss. Additionally, there are no differences in the performance of the Vivacell 100 when concentrating either soluble or membrane proteins.