

## Determining the most Effective Dialysis MWCO for Protein Purification

### Background:

Spectrum offers low protein-binding Biotech Cellulose Ester (CE) Dialysis Tubing and Float-A-Lyzer® G2 Ready-to-Use Dialysis Devices in 9 concise Molecular Weight Cut-Off's (MWCO) in the range of 100 – 1,000,000 Daltons (D). Since the membrane MWCO is based on the molecular size retained by 90%, the challenge in purification applications is knowing which MWCO is the most effective for removing unwanted low Molecular Weight (MW) contaminants.

Since the ionic species in desalting, pH change and buffer exchange applications are sufficiently smaller than the membrane pores and at least 1000 times smaller than macromolecules, most MWCO's between 3.5 kD and 100 kD will achieve the desired separation within 1-2 days. The only consideration required is that the MWCO should be smaller than the MW of the macromolecular species to be retained.

Dialysis is also a preferred method for the gentle purification of labile proteins, sensitive enzymes and delicate complexes in order to maintain activity, functionality or a stable aqueous environment. However, since the lower MW contaminants to be eliminated are often only 10-100 times smaller than the target protein, not all MWCO's are equally effective in achieving the desired separation. Consequently, selecting the optimal membrane MWCO is much more critical in macromolecular purification applications.

### Objective:

Using Float-A-Lyzer® G2 Dialysis Devices (FAL-G2), determine the relative effectiveness of 20 kD, 50 kD and 100 kD MWCO's to retain antibody (IgG, 166 kD) and eliminate unwanted low MW species 100 times smaller (Vitamin B12, 1.35 kD) and 10 times smaller (Cytochrome C, 12.4 kD) in a 24 hour period.

Secondly, to establish general rules for determining the most effective membrane MWCO for dialysis applications like protein purification.

### Method:

- 3 different MW solutions are prepared representing the target protein (IgG – 166 kD: 1 mg/ml 0.9% PBS), the 1/10X MW contaminant (Cytochrome C – 12.4 kD: 0.2 mg/ml 0.9% PBS) and the 1/100X MW contaminant (Vitamin B12 – 1.35 kD: 0.2 mg/ml 0.9% PBS).
- 9 FAL-G2 units (5 ml size) are loaded with 4 ml of solution as follows: IgG in 20 kD, 50 kD and 100 kD MWCO's; Cyt C in 20 kD, 50 kD and 100 kD MWCO's; and Vit B12 in 20 kD, 50 kD and 100 kD MWCO's.
- Each of the 9 FAL-G2's units are dialyzed for 24 hours at room temperature in 9 separate dialysate reservoirs each containing 1 liter of 0.9% PBS buffer; exchanging the buffer after 3 and 8 hours with fresh buffer.
- At 4 time points (0, 3, 8 and 24 hours), 200 µl test samples are taken from each of the 9 FAL-G2; diluted 1:4 in 0.9% PBS and measured on a UV/Vis spectrophotometer (IgG at 280 nm, Cyt C at 406 nm and Vit B12 at 366 nm) to determine the MW solution concentration in each diluted test sample.
- The undiluted concentrations for all 36 test samples are calculated and compared to the original MW solution concentration before dialysis (time point 0) to determine the percent retention of each MW solute (IgG, Cyt C and Vit B12) by each FAL-G2 MWCO (20 kD, 50 kD and 100 kD) at 3 time points (3, 8 and 24 hours).



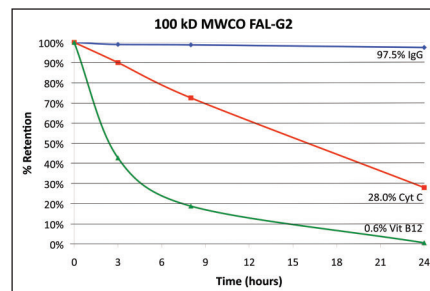
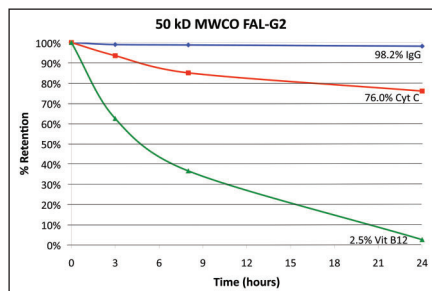
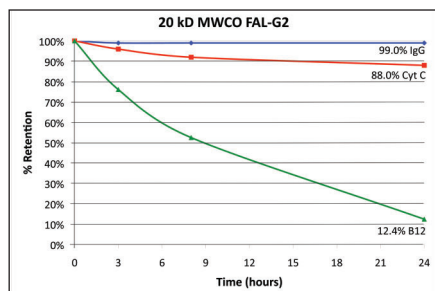
## Results & Discussion:

The Float-A-Lyzer® G2 dialysis device with screw-cap lid makes in-process testing possible so that real-time retention of IgG, Cyt C and Vit B12 are measured at 3, 8 and 24 hours (Table 1) without disrupting dialysis. The percent retentions are compared in Table 1.

A graph for each MWCO (20 kD, 50 kD and 100 kD) is plotted to trend and compare the retention of the target protein, IgG (166 kD); the 1/10X MW contaminant, Cyt C (12.4 kD); and the 1/100X MW contaminant, Vit B12 (1.35 kD).

Solute	Hours	Retention in membrane MWCO's		
		20 kD	50 kD	100 kD
IgG, 166kD (Target)	3	99.0%	99.0%	99.0%
	8	99.0%	98.8%	98.8%
	24	99.0%	98.2%	97.5%
Cyt C, 12.4kD (1/10X)	3	96.0%	93.5%	90.0%
	8	92.0%	85.0%	72.5%
	24	88.0%	76.0%	28.0%
Vit B12, 1.35kD (1/100X)	3	76.1%	62.4%	42.7%
	8	52.5%	36.5%	18.8%
	24	12.4%	2.5%	0.6%

Table 1



All 3 membrane MWCO's (20 kD, 50 kD & 100 kD) retained nearly all of the target protein IgG over 24 hours.

The 20 kD MWCO eliminated most of Vit B12 (87.6%) but only a small percentage of Cyt C (12%).

The 50 kD MWCO removed almost all of Vit B12 (97.5%) but only 24% of Cyt C.

The 100 kD MWCO eliminated essentially all of Vit B12 (99.4%) and also removed the majority (72%) of Cyt C.

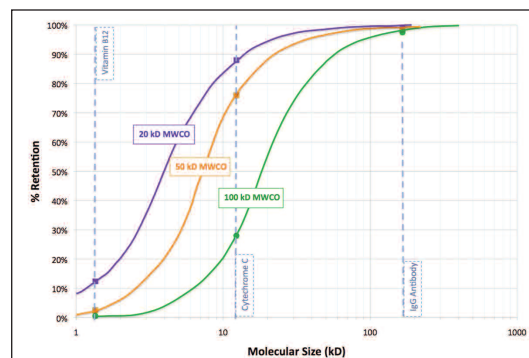
## Conclusion:

The overall relative MWCO effectiveness for IgG purification is: 100 kD > 50 kD > 20 kD; 100 kD being the optimal MWCO for IgG purification.

Referring to the MWCO retention profile curves, the 50 kD and 100 kD membranes effectively eliminate Vitamin B12 (the 1/100X low MW contaminant) in 24 hours; while the 20 kD MWCO would require approximately 36 hours. Only the 100 kD MWCO effectively eliminates Cytechrome C (the 1/10X MW contaminant), however it would require 2-3 days for completion. The 20 kD and 50 kD MWCO's are simply ineffective in eliminating Cytechrome C.

General Rules for Protein Purification by Dialysis:

1. Dialysis requires that the target protein or macromolecule is at least 10X larger, ideally 100X larger, than the contaminants to be removed.
2. The most effective MWCO for protein purification is the highest MWCO just below the size of the protein to be retained.
3. To achieve purification in 1-2 days, the membrane MWCO should be at least 100X larger than the contaminant MW.
4. To achieve, purification in 2-3 days, the membrane MWCO should be at least 10X larger than the contaminant MW.



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