

## Application Note

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### **Vivaflow® 200: A critical sample preparation tool for concentrating hybridoma supernatants**

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#### Abstract

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This study focusses on the use of Vivaflow® 200 crossflow cassettes to concentrate up to 3 L clear murine hybridoma supernatant 10-fold prior to affinity chromatography. Consistent antibody recoveries in excess of 98 % at speeds of concentration around 20–25 mL/min are observed, using two Vivaflow® 200 (30 kDa MWCO) cassettes operating in parallel.

## Introduction

Monoclonal antibodies produced by hybridoma technology are commonly used in various applications in the (bio-) pharmaceutical industry, in research and for in-vitro-diagnostic (IVD) manufacturer. Analytical techniques like western blot, immunofluorescence and ELISA are widespread utilizations. Production and processing of monoclonal antibodies especially for users in the IVD market require high quality measures for example by implementation of an ISO 9001 quality system and state of the art technology.

An integral step in manufacturing of antibodies is filtration by using ultrafiltration and microfiltration membranes (1, 2). The stirred cell used to be the most common laboratory method for concentrating volumes less than 500 mL. Common issues encountered were slow speed, excessive foaming and lack of dead stop causing significant antibody losses in the past. This forced production scientists to look for lab scale tangential flow filtration (TFF) options.

TFF with microfiltration or ultrafiltration membranes is widely used for clarifying, concentrating or buffer exchanging proteins and antibodies (3, 4). The feedstock flows across the filter membrane (tangentially). In ultrafiltration mode, low molecular weight molecules pass through the membrane into the filtrate whereas the feedstock, containing the target antibody, can be concentrated continuously to relatively high protein concentrations in a short time.

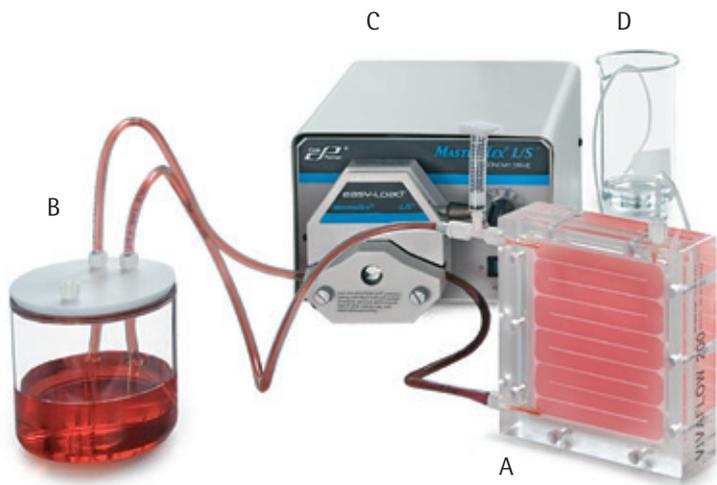


Figure 1: Vivaflow<sup>®</sup> 200 tangential flow cassette (A) with sample reservoir (B), peristaltic pump (C) and receiving flask for filtrate (D).

Robust, reliable and reproducible sample preparation such as protein and antibody concentration | buffer exchange is a fundamental step in downstream processing of all bioreagents. At BioservUK Vivaflow<sup>®</sup> 200 flip flow cassette has been the preferred concentrator for clarified hybridoma supernatant feedstock volumes up to 3 L prior to Protein A or Protein G preparative chromatography. The following parameters were considered when selecting an ultrafiltration concentrator: quality of the UF membrane, speed of concentration, antibody recovery, re-usability and cost-effectiveness.

In this work, we describe the cross flow process applied at BioservUK for concentration of different murine subclasses of IgG monoclonal antibodies.

## Materials & Methods

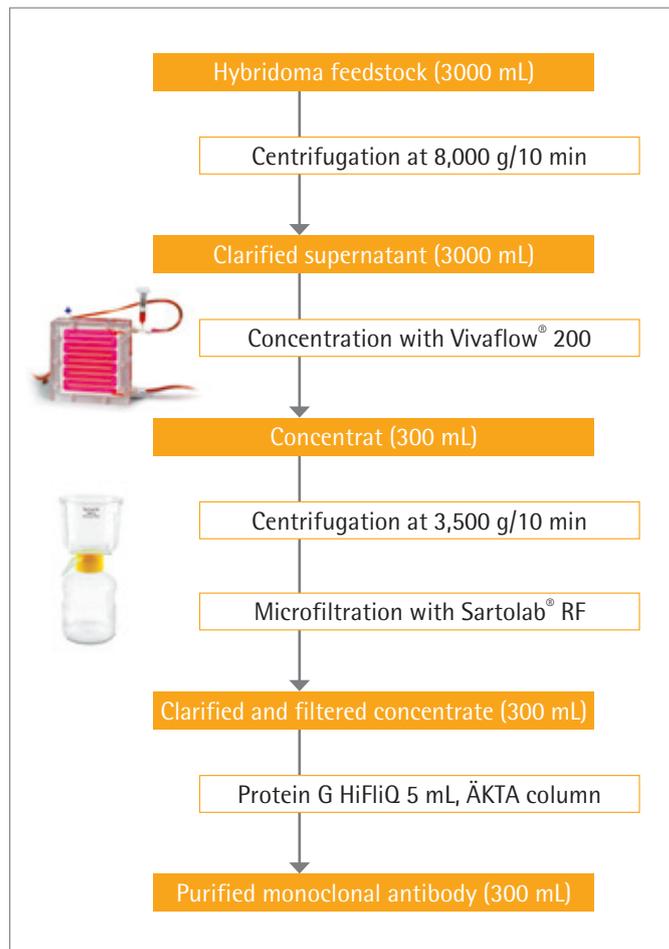


Figure 2: Downstream Processing Workflow of 3 L hybridoma feedstock at BioservUK.

Supernatant is derived from hybridoma cells formed by fusion of murine B-lymphocytes with the Sp2.0 mouse myeloma cell line, grown in roller bottle culture. The hybridoma cells are routinely cultured in RPMI 1640 media (Gibco cat no: 21875) supplemented with 10% EU approved FBS (Gibco cat no: 10270-106). One feedstock is clarified by centrifugation at 8,000 g for 10 min at 4 to 8 °C. The VF200 is pre-washed with 2 L water to remove storage buffer. The integrity of the Vivaflow<sup>®</sup> 200 is checked during the pre-wash step over 1 min to ensure a filtrate flow rate > 55 mL/min.

The supernatant is then concentrated 10-fold with two Vivaflow<sup>®</sup> 200 (30 kDa MWCO, PES, Sartorius cat no.: VF20P2) connected in parallel and using a Type 15 pump head (Fig. 1). The concentration of antibody in cell culture is typically around 30 mg/L. The typical antibody concentration post Vivaflow<sup>®</sup> 200 will be approx. 300 mg/L (10-fold concentration). The maximum flow rate through the Vivaflow<sup>®</sup> 200 concentrators is dictated by the 3 bar pressure limit. After antibody concentration,

the cassettes are flushed with 2 L water followed by cleaning-in-place (CiP) using 500 ml 0.5 M NaOH and 1% sodium hypochlorite in a 40 min. re-circulation mode cycle. The cassettes are then flushed with 2 L water and finally 200 ml 20% ethanol for storage at 2–8 °C. 300 mL concentrate is centrifuged to remove any further particulate at 3,500 g for 10 min and then 0.22 µm filtered with a 500 ml Sartolab RF vacuum filter (Sartorius cat no: 180C2-E) prior to Protein G HiFluQ 5 mL column purification (Protein Ark cat no: HiFluQ5-PG-5).

## Findings

The Vivaflow® 200 is embedded in our SOPs for all our 1–3 L concentration steps. It is incredibly easy to use and has shown consistently >98% antibody recoveries at speeds of concentration around 20–25 mL/min. Crucially, a non-recoverable hold-up volume less than 1 ml is ideal and even the pressure indicator is flushed during the washing steps. The complete, self-contained system can be easily attached to and detached from the Masterflex Easy-Load peristaltic pump head, it can be stored in a fridge and is ready for re-use with no manual adjustment. It is a hands-free and no-mess operation.

We monitor flow rate every 20 min by measuring the filtrate volume at a constant peristaltic pump speed. This corresponds to a setting of 3 on the Masterflex L/S Economy Drive peristaltic pump. Typical flow rates are observed in Figure 4 and varied between 20–25 mL/min. Typical time to concentrate 3 L 10-fold was 2 hour.

At BioservUK we established the Vivaflow® system for different immunoglobulin G (IgG) subclasses. Recoveries of all murine subclasses of IgGs (IgG1, IgG2a, IgG2b, IgG3) are in excess of 98%. We perform a CiP between each 3L preparation. Individual Vivaflow® 200 cassettes are only used for one particular IgG subtype only. Our SOP permits a maximum 50 L throughput per cassette.

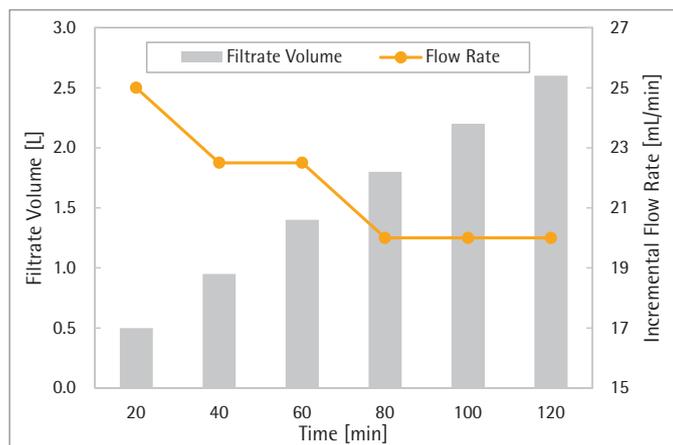


Figure 3: Exemplary flow rate measured as a function of filter volume over time

## Conclusion

We have pivoted our downstream processing of 1–3 L hybridoma cultures around the Vivaflow® 200 tangential flow filtration concentration step because of its ease of use, fast flow rates, low cost, high throughput, broad chemical compatibility and overall ruggedness. In the stirred cell, the horizontal flat membrane disc used resulted in caking of immunoglobulin G on the surface of the membrane and therefore significant flow rate reductions and IgG losses of up to 40%.

We concluded that the Vivaflow® 200 is a unique consumable in lab scale TFF and these crossflow cassettes have proved to be an indispensable tool for concentrating all subclasses of monoclonal IgGs. The Vivaflow® 200 remain our preferred instrument of choice for sample volumes up to 3 L and is considered a monoclonal antibody-grade UF concentrator.

## References

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## Disclaimer

Experiments and Methods have been conducted independently and the authors are responsible for all content in this article.

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