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Maximizing Recovery of Non-Standard IgG Formats Using Rapid Clear® Cap 3000

Rapid Clear® Cap 3000 (P/N: 788116)

Abstract

The Thomson Rapid Clear® Cap 3000 (RC3K) is a diatomaceous earth (DE)–containing depth filtration solution used in bioprocessing and biomanufacturing to clarify liters of cell culture for downstream purification of standard IgG antibodies. When processing **non-standard IgG formats** (e.g., bi-specifics, ADCs, multi-specifics) or certain **antigens**, exposed surface charges can increase the likelihood of non-specific, charge-mediated retention within DE-containing filter matrices.

This protocol introduces a simple post-filtration, high-salt PBS wash designed to disrupt weak electrostatic interactions and release any sequestered target protein from the filter substrate. The wash step enables the RC3K to support non-standard IgG format workflows while maintaining high target recovery.

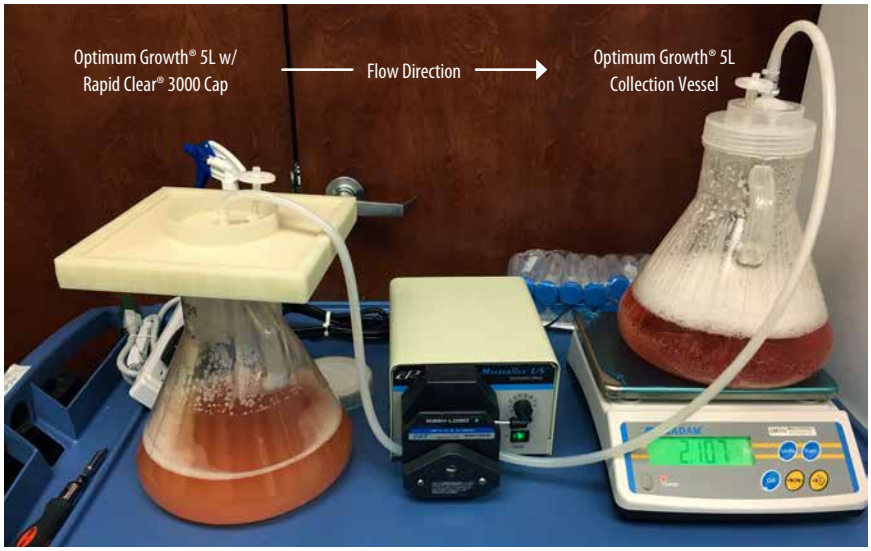
Introduction

The RC3K enables efficient clarification with high recovery across a broad range of molecule types (bi-specifics, ADCs, multi-specifics, antigens, and other engineered formats). In some engineered antibody formats or antigens, particularly those with exposed charged regions, non-specific binding to filtration substrates has been reported. This can result in partial retention of target protein during clarification.

A straightforward way to overcome this is to perform a post-filtration wash that changes the charge environment at the membrane/substrate interface. In this protocol, protein/substrate interactions are neutralized by flushing the filter with high-salt PBS after the initial filtration. The increased ionic strength disrupts weak, charge-driven binding that can occur with engineered antibody formats or antigens, allowing any previously retained (sequestered) target protein to be released from the filter matrix. Collect this wash fraction separately, and pool it with the clarified supernatant as needed before downstream purification or further processing.

Protocol

1. **Assemble the system**
   A. Assemble the Rapid Clear® Cap 3000 onto a sample containing flask and compatible collection vessel.
   B. Route tubing through the peristaltic pump according to your standard setup.
2. **Clarify whole cell culture**
   A. Load up to 3L of whole cell culture using a peristaltic pump.
   B. Expect ~300mL dead volume.
   C. Collect clarified supernatant into a separate vessel (options include 1.6L, 2.8L, 5L and 7L flasks).
3. **Key step: Post-filtration high-salt PBS wash**
   A. After filtration is complete, wash the filter a minimum of 500ml of PBS containing high salt (e.g., 250–500 mM salt, or as specified by your workflow).
   B. Collect the wash fraction. This wash contains any target protein that was previously retained via charge interactions. The most common salt used for this method is NaCL. Other salt forms such as NaK, and NaPO can also be substituted based on compatibility or optimization studies.
4. **Proceed to downstream processing**
   A. Clarified supernatant (and the recovery wash fraction, if combining) is ready for purification (e.g., chromatography) or further processing.



Rapid Clear® Cap 3000 used on a Thomson 5L Flask (P/N: 931116-DB)

Key Features

- **The high-salt PBS wash is the critical recovery step.**
  This post-filtration wash introduces a charge environment that disrupts electrostatic protein/substrate interactions and releases any retained target molecule.
- **Improves recovery for charge-sensitive molecules.**
  Particularly helpful for engineered antibody formats and antigens that may exhibit non-specific, charge-mediated retention.
- **Enables RC3K for broader molecule coverage.**
  Supports secreted antibody formats beyond standard IgG without requiring major changes to existing clarification workflows.

Conclusion:

The RC3K system streamlines liter-scale clarification by replacing traditional multi-step vacuum bottle-top filtration workflows with a single-step, cap-based process. This simplifies handling, reduces transfers and consumables, shortens processing time,

and improves overall workflow efficiency at the liter scale. Adding a post-filtration high-salt PBS wash extends RC3K performance to all molecule types, including non-standard IgG formats and antigens that may otherwise experience charge-mediated retention. Laboratories can integrate non-standard formats into RC3K processing with minimal disruption while preserving high target recovery.

Note: Investigators should understand the effect of high salt concentrations on target proteins prior to attempting this protocol.

CELL LINE VIABILITY	99%-70%		69%-50%		49%-40%		39%-0% SPIN FOR 7MIN @ 4000G*	
CELL TYPE	VOLUME (L)	TIME (MIN)	VOLUME (L)	TIME (MIN)	VOLUME (L)	TIME (MIN)	VOLUME (L)	TIME (MIN)
CHO Stable without Feed	3.0	18	2.5	18	2.0	20	3.5****	35****
CHO Stable, 1 to 2 Feeds	2.0	18	2.0	18	1.5	35		
CHO Stable, 2+ Feeds	Spin for 7 min @ 4000g; ≤3L volume ****							
HEK293 (FreeStyle™ & Expi293)	3.0	18	3.0	23	3.0	25	3.5****	35****
CHO Transient	3.0	18	2.5	18	1.5	35		
ExpiCHO	3.0	18	2.5	18	1.0	18		

Appendix:

Viability and Density effects on cap performance

* For low viability cultures, (< 39%), centrifuge for 7 minutes prior to clarifying with the Rapid Clear® Cap.
** This chart was created from results generated in customer labs.
*** All data was generated using a Cole-Parmer pump (pump drive p/n EW-07554-90, pump head p/n EW-77200-62)
**** Cell cultures that received 2+ feeds will require spinning to minimize potential clogging