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Lab-Scale Harvest and Clarification of Lentiviral Vectors Using Sartoclear Dynamics® Lab Filtration Systems

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Abstract

In this study, the use of Sartoclear Dynamics® Lab V50 (Sartorius) comprising the diatomaceous earth (DE) filter aid was evaluated for clarification of lentiviral vectors (LV) produced by HEK293 suspension cells. The effect of the DE concentration and incubation time on filtration time, yield and turbidity reduction was analyzed in a design of experiment (DOE) approach. Moreover, the use of Sartoclear Dynamics® Lab V50 was compared against the standard laboratory method (centrifugation and subsequent filtration) to examine if DE can effectively clarify LV as this filter aid is easy to handle. Using the DOE approach, we found that higher DE concentrations are advantageous for higher turbidity reduction and faster filtration times, but that lower DE concentrations are advantageous for recovering high LV titers. Compared with the standard method, Sartoclear Dynamics® Lab V50 filtration systems resulted in better turbidity reduction, greater removal of contaminants and higher filter capacities.

Introduction

With the advent of gene and gene-modified cell therapy, viruses have become critical tools in medicine. Gene therapy has significant therapeutic potential for the treatment of many hereditary and acquired diseases [1], with three thousand clinical trials with viral vectors registered as ongoing, among which about 10 % are conducted with lentiviral vectors [2]. Over two thousand unique gene-modified cell products are currently in development, and four CAR-T therapies have already been approved to address oncology indications. The promising prospect of the ongoing clinical trials on gene and gene-modified cell therapy [3, 4] means that there is a strong demand for scalable and cost-effective methods for production and purification of viral vectors [5].

Lentiviral vectors (LV) are typically produced by transient transfection of HEK293T cells with multiple vector plasmids [6, 7]. The virus is released into the supernatant; hence, the cells must be removed from the cell culture broth. One of the challenges in CAR-T research is the lack of an established method for LV clarification that is considered the gold standard. The aim of the clarification process is to eliminate major contaminants and to reduce turbidity of the solution, while maintaining virus activity. Laboratory-scale clarification is typically performed by centrifugation of the fermentation broth and supplemented by subsequent microfiltration of the supernatant [8, 9, 1]. Simplifying harvest and clarification by using only single-step membrane filtration, which can be achieved through the use of filter aids, would improve the efficiency of LV purification and T-cell transduction.

To simplify CAR-T research workflows, Sartorius T-Cell Screening Solution offers a semi-automated multiplexed way for the discovery of novel targets and development of efficient CAR constructs (Figure 1). In particular, the Sartoclear Dynamics® Lab series of filtration products utilized for clarification of cell culture broth were developed to facilitate a rapid and efficient viral vector harvest workflow and are based on the principle of dynamic body feed filtration. The diatomaceous earth (DE) filter aid consists of fused skeletal remains of diatoms with a highly porous structure [10]. After the upstream process, the culture broth is mixed with a filter aid first, then applied to a filter membrane. DE forms a nearly incompressible porous cake, and its high porosity allows liquid to flow around the particles, preventing filter clogging [11]. The use of filter aids eliminates the centrifugation step and reduces processing time.

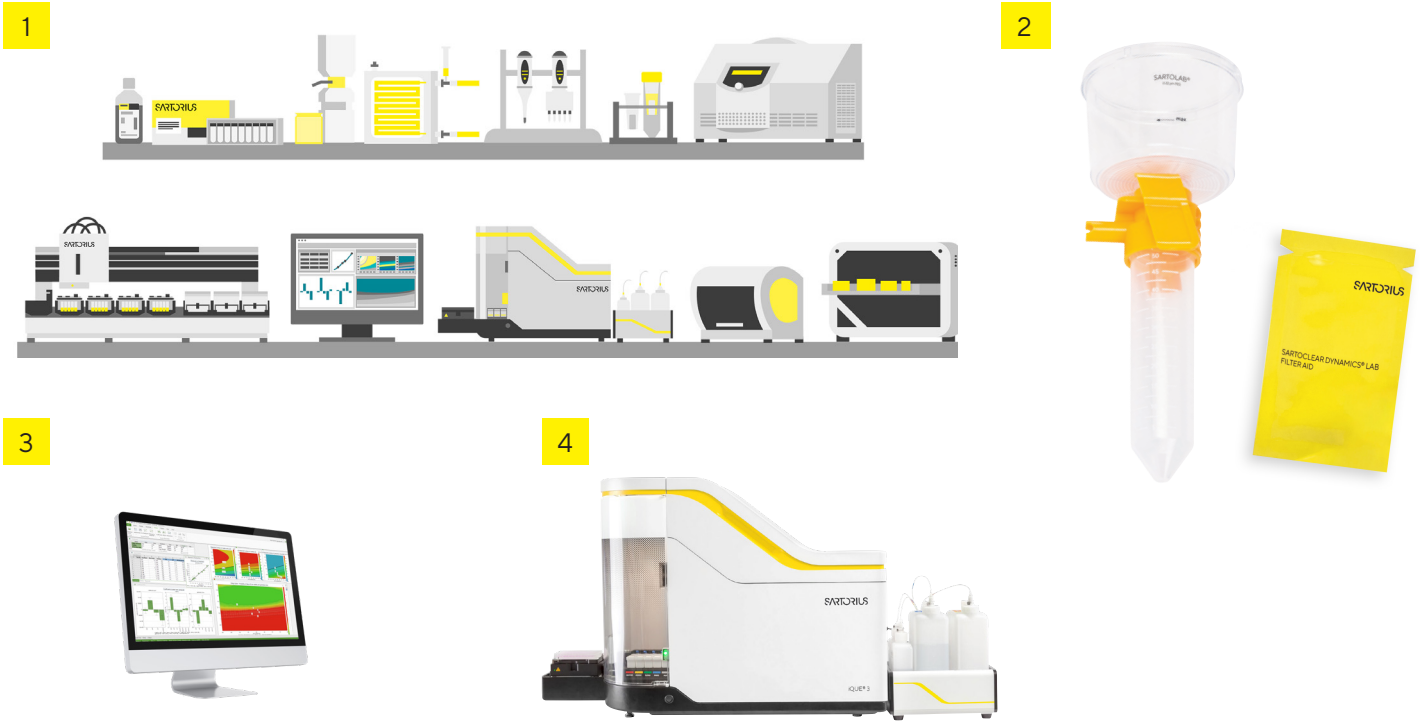

Figure 1: Sartorius T-Cell Screening Solution (1), Sartoclear Dynamics Lab® V50 (2), MODDE® (3), and iQue® 3 (4).

Materials and Methods

The lentiviral vector was expressed by transient transfection of HEK293T/17 SF cells, cultivated in suspension using the UniVessel® single-use 2 L bioreactor (Sartorius), with four plasmids encoding the essential lentiviral genes, as described in detail by Labisch et al. [12].

Clarification of 50 mL of LV culture broth samples was performed using the Sartoclear Dynamics® Lab V50 0.45 µm polyethersulfone (PES) version (Sartorius). The Sartoclear Dynamics® Lab V50 kit contains Sartolab® RF50 vacuum filtration units, which are comprised of a funnel, a 50 mL conical tube and a tube connector for vacuum connection. The kit is supplied with a choice of standard quantities of 1 g of DE (Sartorius, SDLV-0050-01F0-2) or 2 g of DE (Sartorius, SDLV-0050-02F0-2) for filtration of up to 50 mL. DE concentrations of between 5 g/L to 40 g/L cell culture broth were tested. DE was added to 50 mL of the cell culture broth and mixed to obtain a homogeneous suspension, then immediately passed through the filters. Additionally, clarification of 50 mL cell culture broth was performed without DE in a two-step method of 5 min centrifugation at 800 x g and subsequent filtration through a Sartolab® RF50 with a 0.45 µm PES membrane (Sartorius, 180F01---------2). For filter capacity determination, clarification was performed until filter clogging occurred, and the filtrate volume was determined. Vacuum filtration was performed with the Sartolab® MultiStation (Sartorius, SDLC01), which is a stand specifically designed to hold one to six Sartolab® RF50 vacuum filtration units, allowing simultaneous filtration of up to six samples.

A design of experiment (DOE) approach was performed to optimize the clarification conditions. The DE concentration was varied between 5 g/L, 12.5 g/L and 20 g/L and the DE contact time between 0 min, 10 min and 20 min. The experiments were planned as a full factorial design with four center points and evaluated using the MODDE® software (Sartorius). Analytical parameters employed to determine clarification performance were the following: measurement of turbidity using an Orion™ AQUAfast AQ3010 turbidity meter (Thermo Fisher Scientific); total dsDNA concentration (Quant-iT™ PicoGreen™ Assay, Thermo Fisher Scientific); and total protein concentration (Pierce™ Coomassie Bradford protein assay kit, Thermo Fisher Scientific) before (centrifuged material) and after filtration. Furthermore, the LV particle titer was determined by performing p24-ELISA (QuickTiter™ Lentivirus Titer Kit, Cell Biolabs). The infective lentiviral particle titer was measured by transduction of adherent HEK293T cells with virus samples and measurement of transgene expression (CD19-CAR construct) by flow cytometry with the iQue® Screener PLUS (Sartorius), as previously described [12].

Results and Discussion

Evaluation of the Impact of DE Concentration and DE Contact Time on Turbidity, Infective Titer and Filtration Time in a DOE Study using MODDE®

To investigate the effect of DE on LV clarification, the factors of DE concentration and contact time with DE were selected for a DOE approach to assess the influence of these parameters on turbidity, infective titer and filtration time. The total cell density of the cell culture broth used for the filtration runs of the DOE was 3.7 x 10^6 cells/mL with a turbidity of 382 NTU.

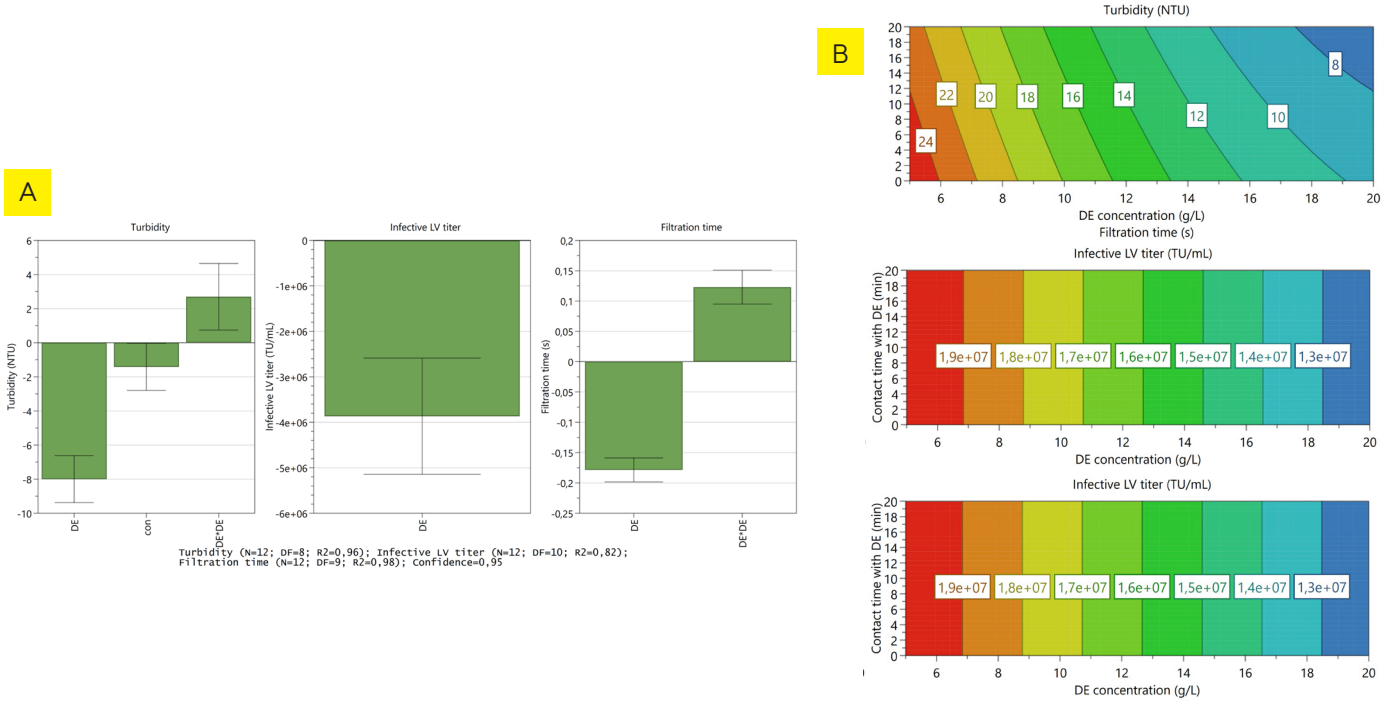

Figure 2: Effects of diatomaceous earth (DE) on lentivirus clarification performance. Regression coefficient plot (A) and response contour plot (B) for the factors of DE concentration (DE) and contact time (con) on the responses comprising turbidity, infectious lentiviral vector (LV) titer, and filtration time.

Figure 2 shows that the responses comprising infectious titer and filtration time are only affected by the factor of DE concentration. As the DE concentration rises, the infectious titer decreases linearly. For filtration time, a quadratic term for the DE concentration exists, resulting in a reduction of filtration time as the DE concentration in the defined concentration test range increases. The turbidity was linearly reduced as the contact time of the sample with DE was extended. Furthermore, with increasing DE concentration, the turbidity decreased, where the latter was the factor with the higher effect.

Evaluation of the Impact of Sartoclear Dynamics® Lab V50 on the Processing Time

The handling of the Sartoclear Dynamics® Lab V50 kit with different DE concentrations was compared to that for microfiltration of the centrifuged supernatant using the Sartolab® RF50 (standard two step method). Upon harvesting of the cell culture, the cell density for the handling experiments was 1.11×10^6 cells/mL and the turbidity was 141 NTU. The entire processing time was broken down into preparation time (i.e., measuring culture volumes, removing supernatant after centrifugation, weighing DE and adding DE to the cell suspension), centrifugation time and filtration time.

During clarification of the centrifuged supernatant, the first Sartolab® RF50 filter became clogged after approximately 25 mL. Hence, a second Sartolab® RF50 filter was required so the remaining unfiltered solution had to be transferred to a second filter, which was laborious and complicated overall handling. Using Sartoclear Dynamics® Lab V50 reduced total preparation time as it eliminates the need for a centrifugation step (Figure 3A). The use of Sartoclear Dynamics® Lab V50 reduced the handling time 3.8 fold compared with the standard method. Filtration time depended on the DE concentration; it was 25 s for 5 g/L and down to 13 s for 60 g/L (Figure 3B).

Evaluation of the Impact of Sartoclear Dynamics® Lab V50 on Turbidity and Filter Capacity

Clarification was performed either with Sartoclear Dynamics® Lab V50 and 5 g/L DE or with the conventional two-step Sartolab® RF50 method involving centrifugation of the cell culture broth and subsequent filtration. The lentivirus containing cell culture broth used for filter capacity determination had a total cell density of 3.7×10^6 cells/mL and a turbidity of 398 NTU at the time of harvest.

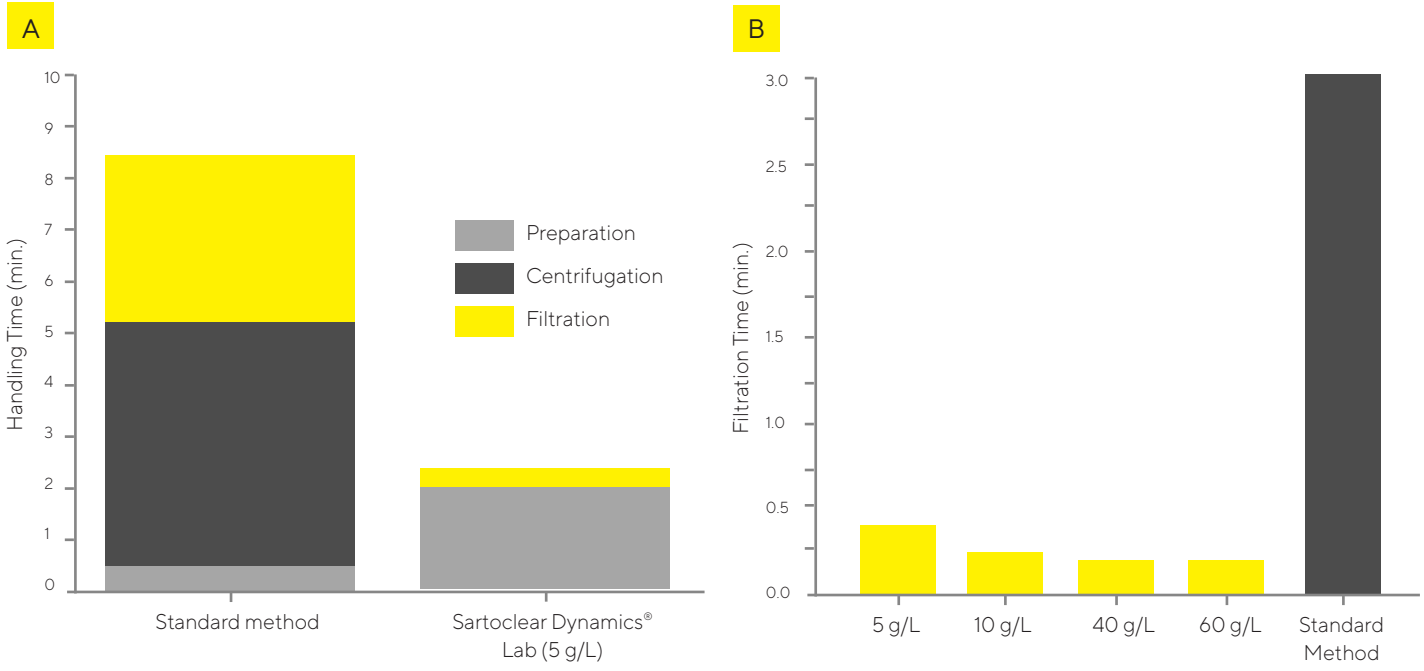

Figure 3: Comparison of clarification methods by handling time (A) and filtration time (B). The preparation, centrifugation and filtration times it took to clarify a 50 mL mammalian cell culture containing LV using the standard two-step method of centrifugation combined with Sartolab® RF50 0.45 µm PES filtration (clogged at a filtrate volume of approx. 25 mL and two filtration units were used) vs. using the one-step method of Sartoclear Dynamics® Lab V50.

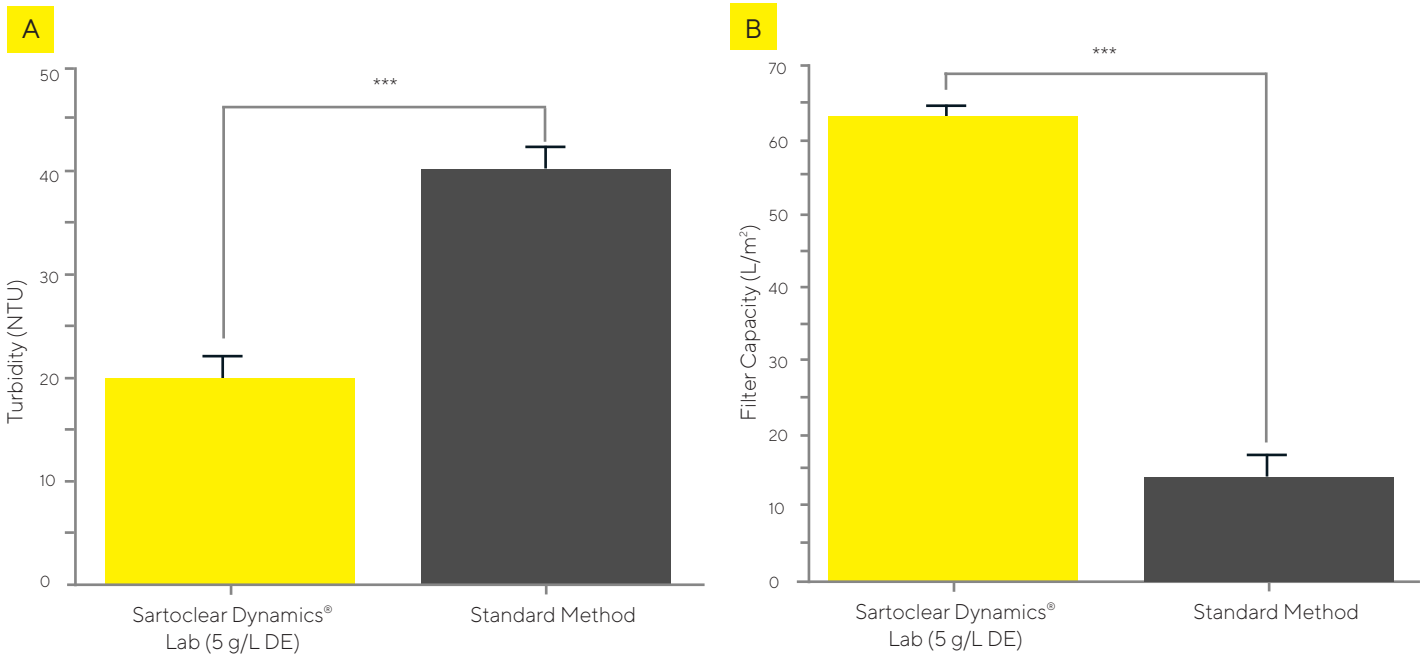

Figure 4: Effects of clarification methods on turbidity (A) and filter capacity (B). Clarification was performed either with Sartoclear Dynamics® Lab V50 with 5 g/L diatomaceous earth (DE) or by centrifugation of the culture broth and filtration through the same microfilter. The LV containing cell culture broth had a cell density of 3.7×10^6 cells/mL and a turbidity of 398 NTU at the time of harvest. Mean ± standard deviation; N = 3; data was analyzed with an unpaired t-test (* $p \leq 5\,\%$, ** $p \leq 1\,\%$, *** $p \leq 0.1\,\%$).

According to Figure 4A, the two-step clarification method combining centrifugation and filtration (standard method) results in a significantly higher turbidity of 43 NTU (89 % reduction), whereas the Sartoclear Dynamics® Lab V50 reduced turbidity by 95 % down to 21 NTU. Filter capacities were determined by using just a minimum DE concentration of 5 g/L with Sartoclear Dynamics® Lab V50 for clarification. Filtration was continued until filter clogging occurred. Performing the conventional lab-scale method of centrifugation for cell removal and clarification of the supernatant resulted in rapid filter clogging just after 33 mL. Using 5 g/L DE as a filter aid increased the maximum filtration volume immensely, enabling the maximum filterable volume of the 50 mL filtration device used to be exceeded by more than two-fold. Filter capacities increased significantly when 5 g/L of DE was used (Figure 4B). By contrast, filter capacities were low when conventional clarification was performed by centrifugation and subsequent filtration (15 L/m²). When 5 g/L of DE was used, filter capacities increased to about 63 L/m². After clarification with DE, the infectious LV titer was found to be typically 75 % or higher compared with centrifuged material.

Evaluation of the Impact of Sartoclear Dynamics® Lab V50 on Removal of Impurities

In a separate experiment, the potential of Sartoclear Dynamics® Lab V50 for removing process related impurities was analyzed by quantification of total protein and dsDNA removal (Figure 5).

A DE concentration of up to 10 g/L DE increased the removal of protein and dsDNA, but higher DE concentrations between 10 g/L and 40 g/L did not lead to any further improvement of impurity removal. In contrast, the use of Sartoclear Dynamics® Lab V50 resulted in significantly higher removal of impurities compared with the standard two-step method of centrifugation and filtration.

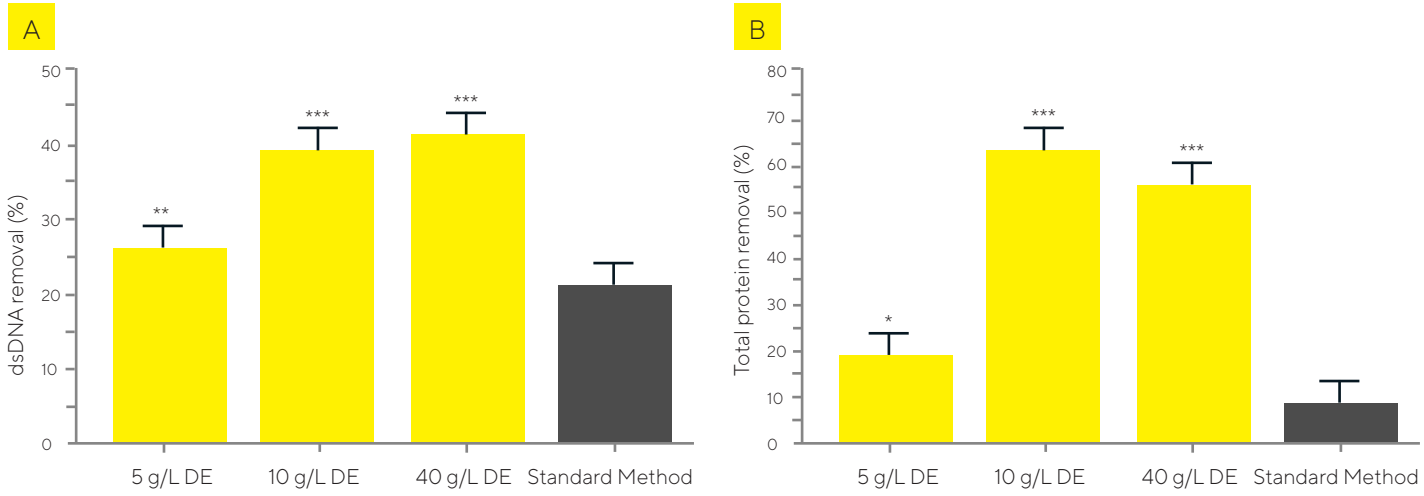

Figure 5: Removal of dsDNA (A) and total protein (B) by different clarification methods. The clarification methods analyzed were clarification using Sartoclear Dynamics® Lab V50 with different DE concentrations (yellow) and centrifugation with subsequent clarification of the supernatant using Sartolab® RF50 (black). Mean ± standard deviation; N = 3; data with and without DE were analyzed with an unpaired t-test (* $p \leq 5\,\%$, ** $p \leq 1\,\%$, *** $p \leq 0.1\,\%$).

Conclusion

Sartoclear Dynamics® Lab filtration systems enable clarification of LV containing mammalian cell cultures without the need for centrifugation. Compared with the standard method, Sartoclear Dynamics® Lab V50 resulted in better turbidity reduction, greater removal of contaminants and higher membrane capacity. This underscores the suitability of Sartoclear Dynamics® Lab filtration systems for harvest and clarification of lentiviral vectors. The DE concentration needs to be adjusted, however, in order to recover high yields of LV and to avoid loss of yield due to an inappropriately high quantity of DE selected. The Sartoclear Dynamics® Lab V50 kit with DE enables the lab technician to perform one-step clarifying filtration that not only facilitates faster and safer handling, but also lowers the quantity of lab materials used. The kit allows an optimized DE quantity to be selected that greatly increases filter capacity, thus ensuring an efficient and a robust lentivirus clarification process in gene and gene-modified cell therapy.

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Abbreviations

CAR	Chimeric antigen receptor
(ds)DNA	(Double stranded) desoxyribonucleic acid
DE	Diatomaceous earth
DOE	Design of experiment
HEK	Human embryonal kidney cells
LV	Lentiviral vector
PES	Polyethersulfone
TU	Transducing units
VP	Viral particles

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