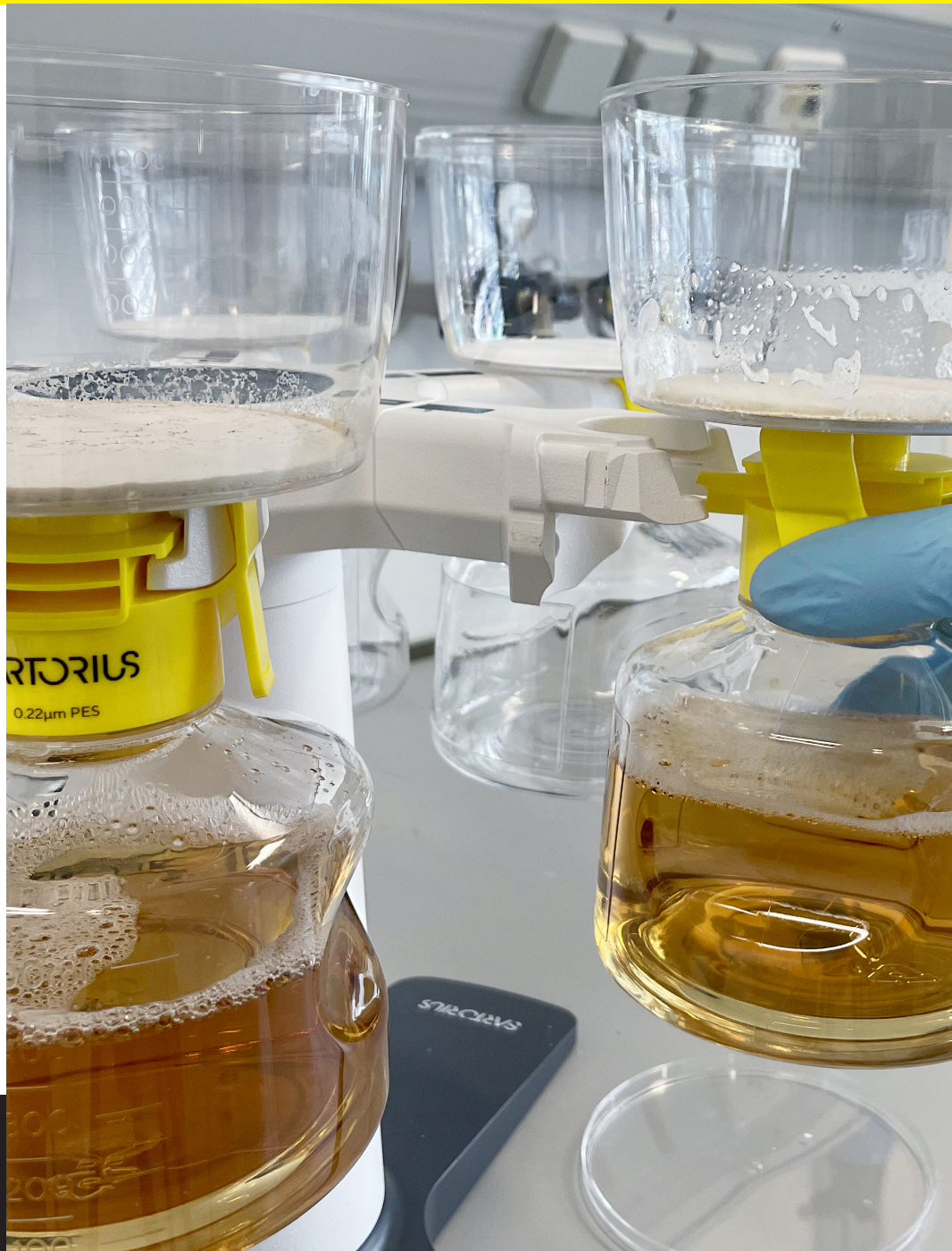


Diatomaceous Earth (DE) – Celpure® C300 Optimal Usage Guidelines for DE Across Various Applications

Diatomaceous Earth (DE) is a naturally occurring substance derived from the fossilized remains of diatoms, tiny, aquatic organisms with intricate silica-based shells. These microscopic entities boast a vast array of unique three-dimensional forms, ranging from perforated discs and spherical shapes to complex structures resembling ladders, feathers, and needles. Their sizes vary from 1 μm to 1 mm, predominantly composed of silicon dioxide (SiO_2).

The highly porous nature and chemical inertness of DE make it an invaluable resource in filtration processes, particularly within the biotechnological sector. Here, DE serves as a crucial component in depth filters designed for cell harvesting, offering exceptional biomass retention capabilities.



In body feed filtration, DE is added to the cell culture broth, where it fulfills a dual role:

- It forms a permeable filter cake, enhancing the overall efficiency and throughput of the filtration process.
- It effectively sieves out sub-micron particles, ensuring a cleaner separation.

To assist you in determining the appropriate amount of DE for your specific application, we have provided a set of guidelines below.

For user convenience, Sartorius supplies pre-packaged, ready-to-use DE pouches in quantities of 1 g, 5 g, 10 g, and 20 g. These pouches contain DE pre-wetted with ultrapure water at a ratio of 1 g DE to 2.25 g water, minimizing dust and ensuring safe handling. Additionally, the pouches are sterilized for immediate use in sensitive environments.

When utilizing a portion of DE from a pouch, it is important to account for the water content by applying the appropriate conversion factor during the weighing process. For instance, to achieve a concentration of 6 g/L DE in 1 L of cell culture broth, one must measure out 13.5 g of the pre-wetted DE (6 g multiplied by the 2.25 conversion factor).

	mAb	AAV	LV	Phages
Typical batch properties at harvest	CHO HEK suspension batch fed-batch process 10 – 30E6 cells/mL; 10 – 90% viability; 0.01 to 5 mg/mL mAb; WCW 40 to 100 g/L	HEK suspension lysate batch process 3 – 4E6 cells/mL; 50% viability; >E12 VP/mL; WCW 10 to 14 g/L	HEK suspension batch process 1 – 5E6 cells/mL; 60 – 70% viability; 3E10 to 2E11 VP/mL; 1E7 to 2E8 TU/mL	<i>E. coli</i> C (ATCC13706), phiX174 phage lysate; WCW 14 g/L
Recommended dry DE weight for 1 L cell culture broth	50% of WCW → 20 to 50 g; up to 80 g for difficult-to-filter samples	50 % of WCW → 5 – 7 g	50% of WCW → 5 – 8 g	30-50% WCW → 5 – 10 g
Converted in pre-wetted DE weight (provided in the pouches)	45 g to 112.5 g; up to 180 g	11.25 g to 15.75 g	11.25 g to 18 g	11.25 g to 22.5 g
DE grade type	C300	C300	C300	C300
Sartolab RF membrane type	0.22 µm PES	0.22 µm PES	0.45 µm PES	0.22 µm PES

Additional Comments for Each Modality:

- mAb:** No titer loss for mAb at high DE levels; Cell viability doesn't predict filterability; Media, supplements, and cell lines greatly affect filterability; 40 g DE/L is sufficient for most processes.
- AAV:** For optimal recovery, use the minimum amount of DE needed for effective filtration; A ratio of 0.181 mg DE per E10 AAV particles should not be exceeded.¹
- LVs:** For maximum recovery, use the lowest effective DE amount for cell broth filtration.²
- Phages:** Use at least 5 g DE/L for results like centrifugation with a 1.5% WCW.

Abbreviations:

- AAV Adeno-associated virus
 CHO Chinese hamster ovary
 DE Diatomaceous earth
 HEK Human embryonal kidney cells
 LV Lentivirus
 mAb Monoclonal antibody
 PES Polyethersulfone
 TU Transducing units
 VP Viral particles
 WCW Wet cell weight

1. A robust and efficient alluvial filtration method for the clarification of adeno-associated viruses from crude cell lysates (ScienceDirect) Centrifuge Free Clarification and Harvest of Adeno-associated Viruses (AAV) (sartorius.com)
2. A new simplified clarification approach for lentiviral vectors using diatomaceous earth improves throughput and safe handling (ScienceDirect) <https://www.sartorius.com/resource/static/celum/109046/Lentivirus-Vacuum-Filtration-CAR-T-Application-Note-en-L-Sartorius.pdf> <https://www.sartorius.com/resource/static/celum/109047/Lentivirus-Sartoclear-Dynamics-Lab-CAR-T-Application-Note-en-L-Sartorius.pdf>

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