Sustainable Alternatives to Triton™ X-100 Detergent for Biomanufacturing: The Deviron® Detergent Portfolio

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Introduction

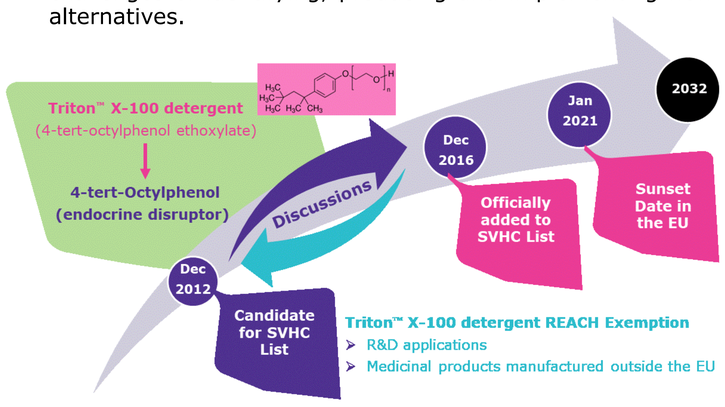
Viral safety is a **major concern for biotherapeutic** manufacturers.

- Cell-based processes may produce endogenous retroviral particles, and adventitious viruses can be introduced from contaminated source materials or during the manufacturing process.
- Human plasma-derived products are at risk of containing viruses, despite extensive screening of donation material.

Detergent-mediated **viral inactivation** is widely used in multiple biotherapeutic production processes as part of an overall virus safety strategy.

Triton™ X-100 detergent is widely used.

- A **degradation product** of Triton™ X-100 detergent is 4-tert-octylphenol, an **endocrine disruptor** hazardous for the environment.
- Triton™ X-100 detergent was classified as "**Substance of Very High Concern SVHC**" in the **REACH** (Registration, Evaluation, Authorization and Restriction of Chemicals) in **2017.**
- The European Chemical Agency (**ECHA**) **prohibited** the **unauthorized use** of **Triton™ X-100** detergent in the **EU** in January **2021.**
- The biotherapeutic industry together is faced with the challenges of identifying, producing and implementing new alternatives.



Minimum criteria for alternative detergent candidates

- Robust viral inactivation of enveloped viruses (4-5 LRV)
- Significant cell lysis performance
- Compliant to operational condition requirements (T, pH,…)
- Minimal impact on protein quality (mAbs, AAV…)
- No impact on other unit operations, no process interference
- Easy to detect and remove
- **Non "substance of very high concern" SVHC**
- **Biodegradable, no harmful degradation products**

For Biomanufacturing, more requirements are creating new challenges

- IPEC-PQG GMP
- High purity
- High volumes
- Cost of goods

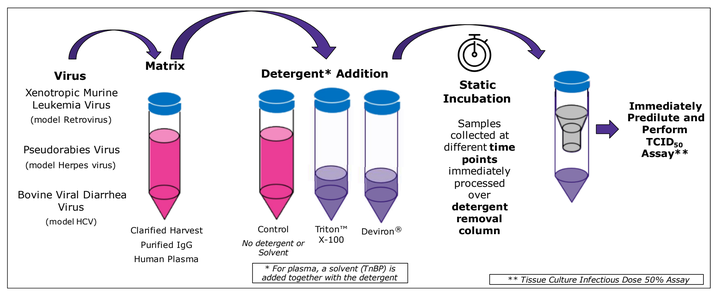
We offer a **portfolio of detergents**, **the** Deviron® detergents, to meet different application and process requirements.

Deviron® Detergents

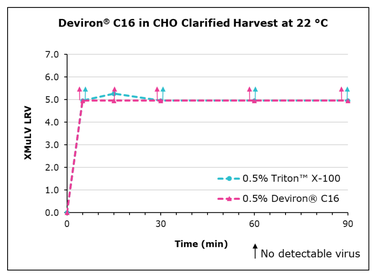
	Deviron® C-16 detergent	Deviron® 13-S9 detergent
Chemical name	N,N-Dimethyltetradecylamin-N-oxide	Alcohols, C11-15-secondary, ethoxylated
CAS number	3332-27-2	68131-40-8
Surfactant	Zwitterionic (pI 8.9)	Non-ionic
CMC	0.002-0.003 wt % (24 °C)	0.005 wt % (24 °C)
Form	30 % wt. water solution	Pure substance (100 % wt.)
Biodegradability (OECD 301B)	Readily biodegradable	Readily biodegradable
Toxicology report	Available	Available
Quality marker	ISO9001	IPEC-PQG GMP
Documentation package	Emprove® Evolve MQ400	Emprove® Expert MQ500
Viral inactivation efficiency	Yes >5 LRV	Yes >5 LRV
Cell lysis efficiency	Yes	Yes
Endotoxin removal for plasmid purification	Yes	Yes
Detection method	HPLC-ELSD method available	HPLC-ELSD method available

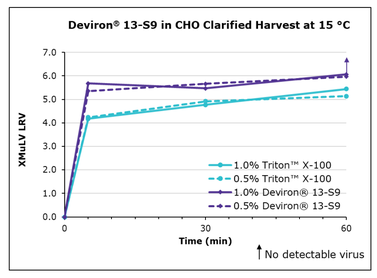
Deviron® Detergents

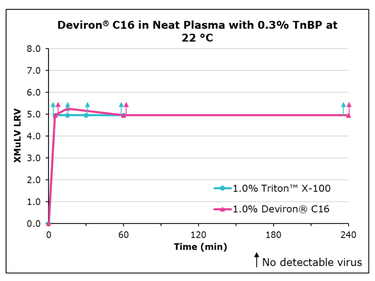
Virus Inactivation

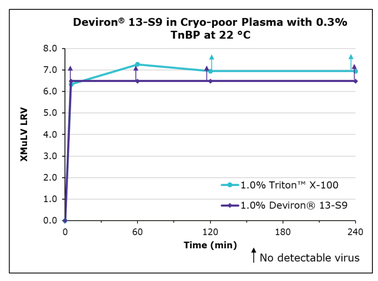


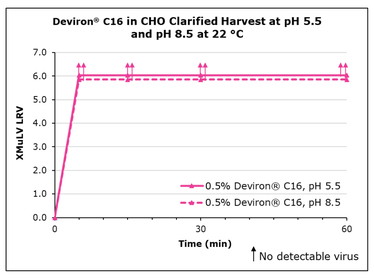
Virus inactivation was assessed in mAb-containing CHO clarified harvest and human plasma matrices with **XMuLV** model.
The standard viral inactivation practice for **mAb processes** is described in the **ASTM E3042-16**. In this procedure the **detergent** concentration is **≥0.5 %, no solvent** (i.e., TnBP) and incubation time ≥60 min.
Plasma processes typically utilize **1.0 % detergent and 0.3 % solvent TnBP** and longer incubation time (4-6 h).
Deviron® C16 and Deviron® 13-S9 detergents demonstrate effective viral inactivation (LRV > 5) in all conditions tested.
Additional data with PRV and BVDV viruses are available.

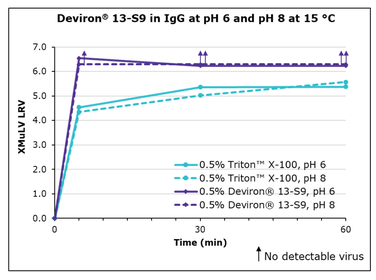






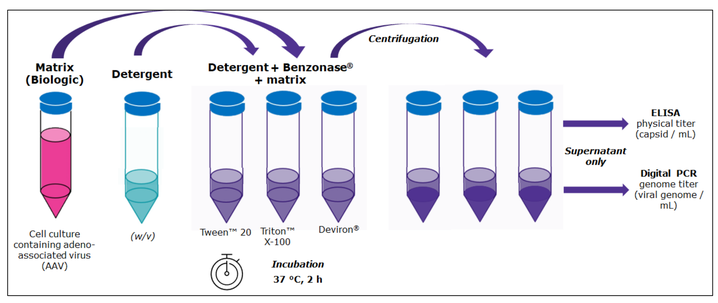


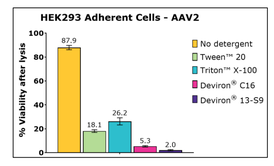


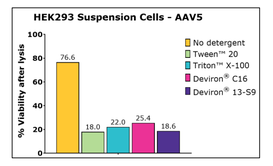


Cell Lysis

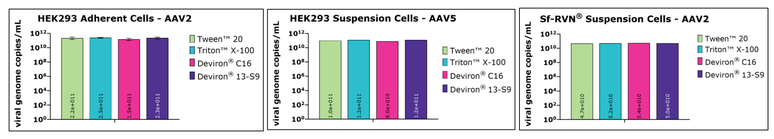
HEK293 and Sf-RVN® cells were cultivated according to their type, suspension or adherent, in the appropriate cell culture media.
HEK293 cells were transfected after one day of culture with the selected plasmid polyethylenimine (PEI) complexation and Sf-RVN® cells infected with Baculovirus at the time of seeding. After cultivation, the cells were lysed.
Detergent: 0.5 % wt.
Nuclease: 25 U/mL Benzonase® endonuclease with 2 mM $MgCl_2$
Lysis time: 2 h
Temperature: 37 °C
Following incubation, total cell count and viability values determined. Virus-containing supernatant was clarified by centrifugation. The supernatant was analyzed to determine physical titer and genome titer. After detergent removal, the AAV infectivity was measured.



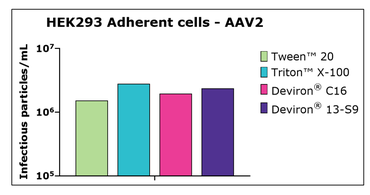




Both Deviron® detergents were excellent in lysing HEK293 and Sf-RVN® cells (data not shown, microscopic images show complete cell lysis). Both Deviron® detergents were comparable or even better than the benchmarks.



Both Deviron® detergents were as efficient as the benchmarks in releasing capsids and the viral genome titers were comparably high.



The infectivity of the AAV is a crucial parameter for final products. Both of the Deviron® detergents preserve infectivity of the AAV after the lysis, as evaluated with the transduction unit assay.

The Deviron® detergents are an advantageous alternative to the benchmarks, combining efficacy, sustainability, and easier handling.

Removal of Deviron® detergents in Downstream Process

Effective detergent removal from a process stream needs to be achieved by downstream steps. Removal was assessed with several different chromatographic resins.

Deviron® C16 detergent

Columns tested	Type of resin	Removal
Protein A	Eshmuno® A	99.6 %
CEX (pH 6)	Eshmuno® S	43-64 %
AEX (bind/elute mode pH 9)	Fractogel® TMAE Hicap	99.8 %
AEX (flow through mode pH 9)	Fractogel® TMAE Hicap	Not suitable

We recommend performing virus inactivation with Deviron® C16 detergent **prior to protein A** affinity capture step

Isoelectric point (**pI**) Deviron® C16 detergent = **8.9**

- pH< 8.9 → net positive charge
- pH>8.9 → net negative charge

Resin selection depends on
- Unit operation pH
- Protein pI

Deviron® 13-S9 detergent

Columns tested	Type of resin	Removal
Protein A	Eshmuno® A	100 %
CEX (pH 6)	Eshmuno® S	99 %
AEX (pH 9)	Fractogel® TMAE Hicap	95 %

Removal analysis performed in **absence of matrix proteins**, to directly assess detergent binding to resin

For specific process and method development, reach out for technical support

Deviron® C16 and **13-S9® detergents** are an effective sustainable alternative to Triton™ X-100 detergent **1**

Deviron® detergents are the result of extensive research and expertise in the field **2**

Selection of a **Deviron® detergent** is based on process and product requirements **3**

Deviron® Detergents

4 Recommendation Ensure compatibility of target protein with detergent

5 Recommendation Evaluate capability of downstream chrom. steps for detergent removal

6 Recommendation optimize detergent detection assay to prevent interference by matrix proteins

The Life Science business of Merck operates as MilliporeSigma in the U.S. and Canada.