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Pharma & Biopharma Raw Material Solutions

Application Note

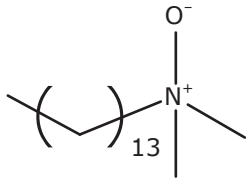
Deviron™ C16 Detergent for Virus Inactivation

Virus clearance is a critical requirement in production of monoclonal antibodies (mAbs) and needs to include multiple orthogonal steps to achieve the required level of safety assurance. In general, a clearance or inactivation step is considered effective or robust if it can achieve ≥4 log reduction of infectious virus.

Typical approaches to virus clearance include detergents, virus filtration and low pH. For many years, Triton™ X-100 detergent (4-tert-octylphenol polyethyloxylate) has been commonly used as a highly effective detergent for virus inactivation. In 2017, however, octylphenol ethoxylates (OPE) were added to the European Authorization list (Annex XIV) of REACH. REACH stands for the Registration, Evaluation, Authorization and Restrictions of Chemicals and addresses the production, import and use of chemical substances and their potential impacts on human health and the environment. REACH affects European manufacturers, importers and downstream users. The REACH Annex XIV sunset date was January 4, 2021. Since this date, OPE products, including Triton™ X-100 detergent, are prohibited in the EU by the European Chemicals Agency (ECHA), unless authorization was granted by the authorities or the intended use is exempt from authorization.

In response to the need for a REACH-compliant detergent for virus inactivation, we identified the Deviron™ C16 detergent (N,N-dimethyltetradecylamine N-oxide; **Figure 1**). This application note describes the use of the Deviron™ C16 detergent for the rapid and robust inactivation of enveloped viruses in clarified mAb harvest. The Deviron™ C16 detergent robustly inactivated all tested model viruses and was shown to be more effective at lower concentration than Triton™ X-100 detergent.

Chemical name:	N,N-Dimethyltetradecylamine N-oxide
Synonyms	Myristyldimethylamine N-oxide, TDAO
CAS No:	3332-27-2
MW:	257.46 g/mol
CMC:	0.14–0.27 mM = 0.0036–0.007 wt%
Stability:	Current information indicates 2 years shelf-life at RT
Biodegradability:	Readily biodegradable (tested according to OECD 301B)



TDAO

Figure 1. Chemical structure and characteristics of REACH-compliant, biodegradable virus inactivation detergent Deviron™ C16 detergent.

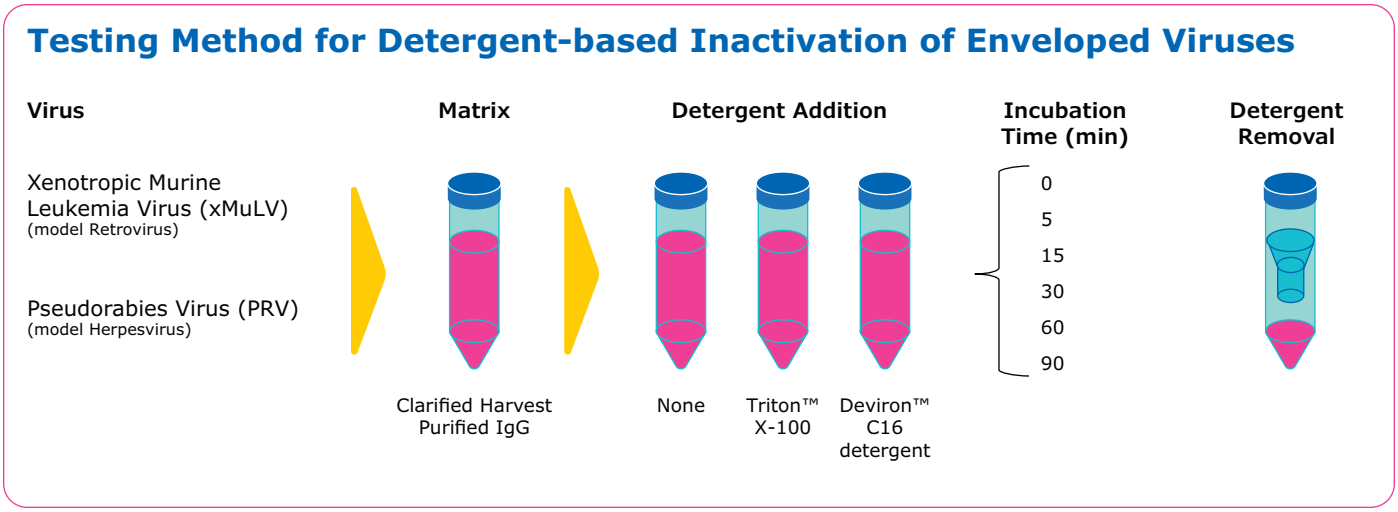


Figure 2. Virus inactivation testing method with Deviron C16 detergent.

Virus Inactivation in mAb Process Clarified Harvest

The Deviron™ C16 detergent was tested for its ability to inactivate two enveloped viruses (Xenotropic Murine Leukemia Virus or xMuLV and Pseudorabies Virus or PRV in clarified harvest or a solution of purified IgG; the impact of pH and temperature on inactivation with Deviron™ C16 detergent was also evaluated. A summary of the testing method, which included parameters specified in ASTM E3042-16 (Standard Practice for Process Step to Inactivate Rodent Retrovirus with Triton™ X-100 Treatment) is provided in **Figure 2**.

xMuLV is a rodent retrovirus and provides a relevant model for retrovirus-like particles that are endogenous to Chinese hamster ovary (CHO) cells, which are commonly used in the manufacture of mAbs. Pseudorabies virus is another enveloped virus, and models Herpesvirus.

In the following studies, model viruses were introduced individually into CHO harvest clarified by passage through a 0.2µm filter (per the ASTM 3042-16 standard) or a solution containing purified IgG. The Deviron™ C16 detergent or Triton™ X-100 detergent was added to reach the indicated final concentration. The impact of pH and temperature on virus inactivation was determined for several virus/matrix combinations. Detergent was removed using chromatographic resin detergent-removal columns, and a $TCID_{50}$ (Tissue Culture Infectious Dose 50%) assay measured the presence of any remaining infectious virus.

Figure 3 is a comparison of Triton™ X-100 detergent and the Deviron™ C16 detergent inactivation of xMuLV in clarified harvest. For this and subsequent figures, the panel on the left shows the reduction in virus titer over time while the panel on the right shows the log reduction value (LRV). The Deviron™ C16 detergent met the ASTM E3042-16 assurance of ≥4 log_{10} of rodent retrovirus inactivation obtained with Triton™ X-100 treatment within a hold time of 60 minutes. No infectious virus was detected in samples treated with the Deviron™ C16 detergent within 5 minutes.

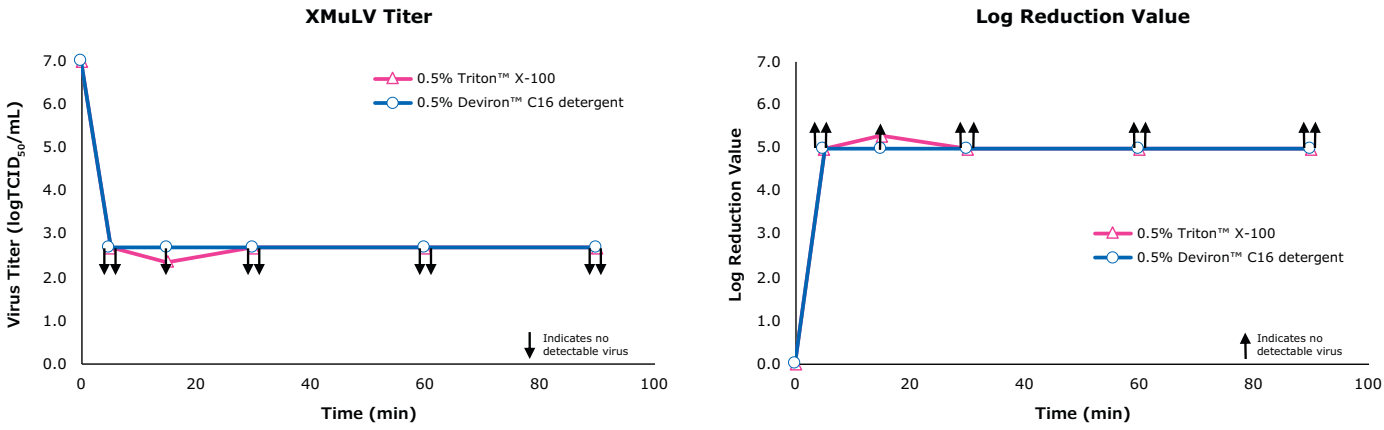


Figure 3. Comparison of Triton™ X-100 detergent and Deviron™ C16 inactivation of xMuLV in clarified harvest.

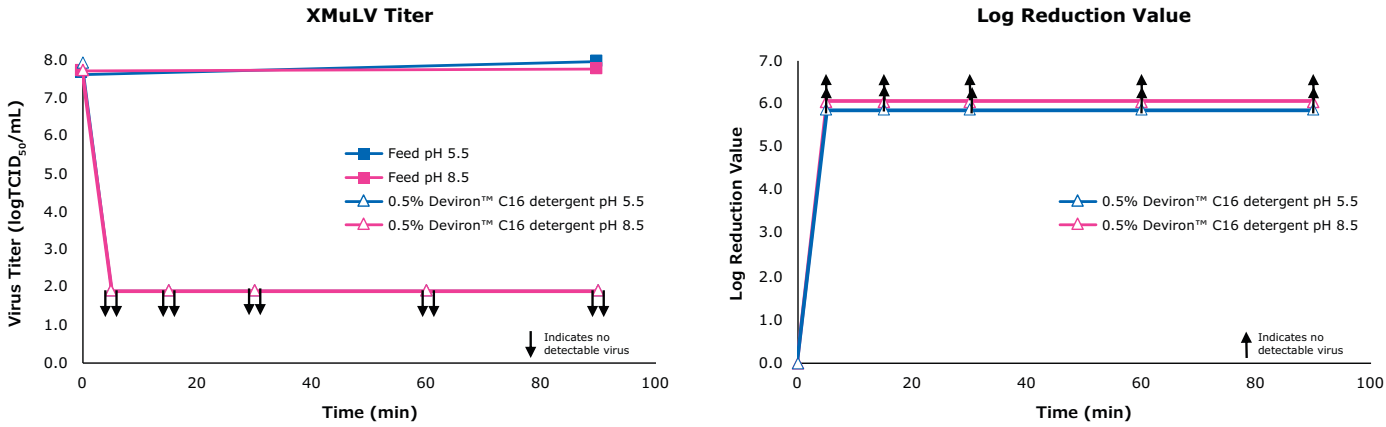


Figure 4. Deviron™ C16 detergent inactivation of xMuLV in clarified harvest over a pH range of 5.5 to 8.5.

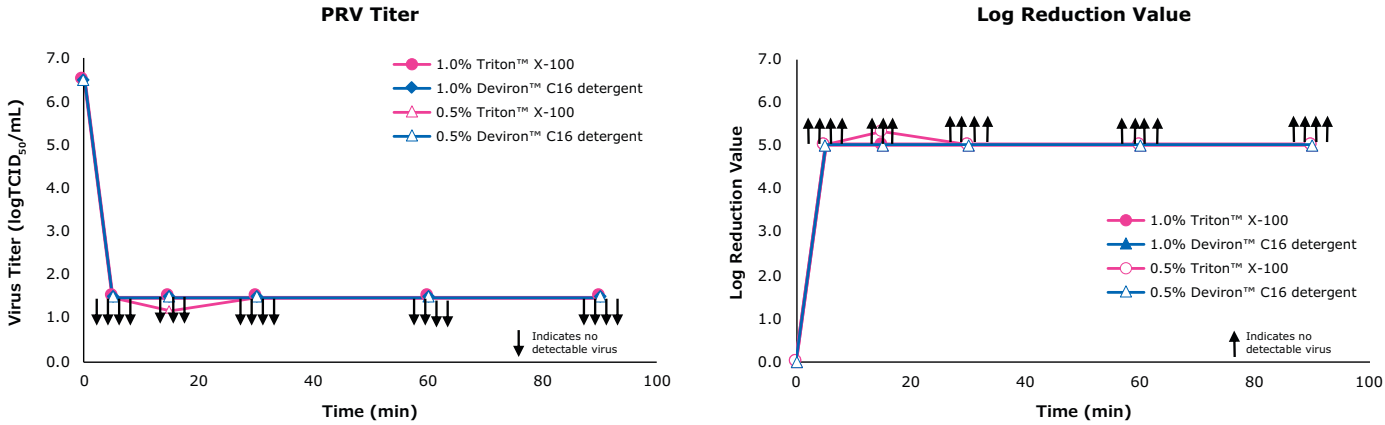


Figure 5. Deviron™ C16 detergent inactivation of PRV in clarified harvest.

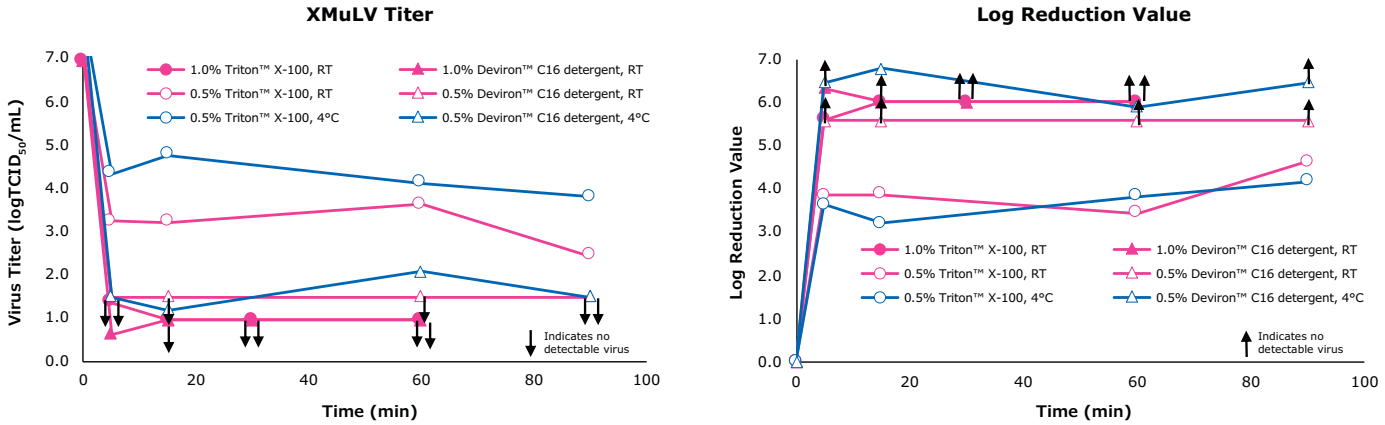


Figure 6. Deviron™ C16 detergent inactivation of xMuLV in purified IgG.

In a similar study, >4 $\log_{10}$ reduction in xMuLV was observed in clarified harvest at pH 5.5 and 8.5 following treatment with 0.5% (w/v) Deviron™ C16 detergent. These data show that Deviron™ C16 detergent inactivates rodent retrovirus across the pH range specified in ASTM E3042-16 (pH 6.0–8.0) (**Figure 4**).

The Deviron™ C16 detergent was as effective as Triton™ X-100 detergent in the inactivation of PRV (**Figure 5**). No infectious virus was detected following 30 min of treatment with 0.5% or 1.0% of either detergent.

Figure 6 demonstrate the ability of Deviron™ C16 detergent to inactivate xMULV at 4 °C, which would be considered a worst-case scenario. xMuLV inactivation with the Deviron™ C16 detergent in purified IgG met or exceeded the level of inactivation achieved by Triton™ X-100 at similar concentrations at each incubation temperature.

Together, these results demonstrate that the Deviron™ C16 detergent was comparable to Triton™ X-100 detergent in the ability to inactivate a range of model viruses and delivered superior performance at low detergent concentration.

Conclusion

After January 4, 2021, Triton™ X-100 detergent was prohibited in the EU by the European Chemicals Agency (ECHA), unless authorization was granted by the authorities or the intended use is exempted from authorization. As a replacement for Triton™ X-100 detergent, the Deviron™ C16 detergent is a biodegradable, REACH-compliant detergent and highly effective at a concentration of 0.5%, typically showing reduction of virus infectivity to non-detectable levels in as little as five minutes.

In virus inactivation studies conducted under conditions specified in ASTM E3042-16, the Deviron™ C16 detergent achieved a 5-6 log reduction of xMuLV at a detergent concentration of 0.5%, with no detectable virus present after five minutes, at both ambient (19–22 °C) and cold temperature (4 °C) and over a pH range of 5.5–8.5. In all conditions tested, the Deviron™ C16 detergent virus inactivation performance was comparable or superior to that of Triton™ X-100 detergent.

The Emprove® Evolve program provides comprehensive documentation about fit-for-purpose high-quality raw materials used in early stages of pharmaceutical and biopharmaceutical manufacturing such as the Deviron™ C16 detergent.

Given this evolving environment, the Emprove® Evolve product line fills the gap between lab-grade and GMP-compliant raw and starting materials. Its purpose is to support quality risk assessments with detailed documentation and supply chain transparency and control, helping customers meet developing regulatory requirements for critical raw materials.

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