MACS GMP Nuclease A: New clinical-grade endonuclease specifically developed for highly reliable production of retroviral vectors in the manufacturing of cell and gene therapy products

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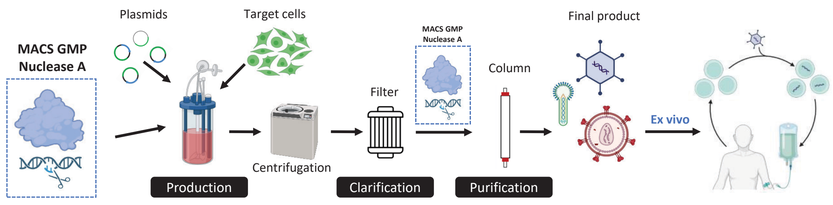
Introduction

The generation of lentiviral vectors used in the context of cell product manufacturing and field of gene therapy poses strict requirements with regard to the purity of the vector. Lentiviral vectors are released into the supernatant of transfected HEK293T cells. This process is prone to host cell nucleic acid as well as plasmid DNA contaminations.

Typically, non-specific endonucleases are used to remove these impurities during the virus purification process, however, these enzymes themselves bear the potential to additionally transmit host cell protein (HCP), DNA or endotoxins to the vector.
Furthermore, endonucleases are widely applied in the beginning of downstream processing (DSP) procedures of biologics to reduce viscosity, enabling easier handling and more efficient protein extraction through the removal of DNA and RNA.
We have therefore developed a clinical-grade endonuclease A, the MACS GMP Nuclease A, combining all required features necessary to be applied for the manufacturing of especially cell therapy products.

Figure 1

Example of Application of **MACS® GMP Nuclease A** in **C**ell and **G**ene **T**herapy



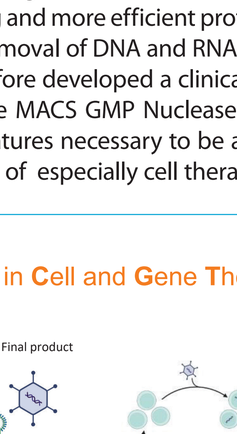
Product overview

1 Strict specifications for high quality standards

Figure 2

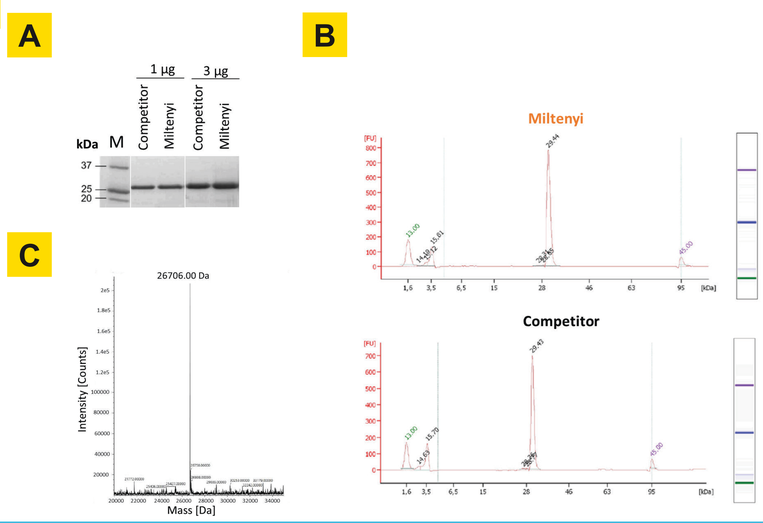
	Specification
HCP*	≤ 10 µg/mg
Endotoxin	≤ 0.25 EU / 1000 U
Nickel content	≤ 5 ppm
Purity	≥ 99 %
Molecular mass	26706 Da ± 6
Specific Activity	≥ 500,000 U/mg
Activity	≥ 250 U/µl
Sterility test	Tested for sterility acc. Ph. Eur.
AOF**	yes
GMP guidelines	ISO 13485, USP <1043>, Ph. Eur. Chapter 5.2.12

*HCP = host cell protein
**AOF= animal origin-free manufacturing



2 Highest purity and LC-MS identity confirmation

Figure 3



A) SDS-PAGE analysis of different protein amounts of the MACS GMP Nuclease A separated on a 4 – 20% SDS gel. 1 µg and 3 µg of protein in comparison to competitor product showed a purity ≥ 99%.

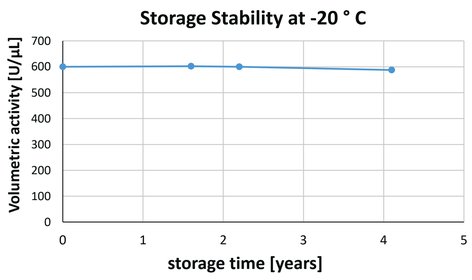
B) Chip electrophoresis analysis with electropherograms and corresponding in-gel-images of the nucleases in comparison [600 µg/mL].

C) LC-MS intact mass determination deconvoluted spectra between 3-4 min of a sample shows exactly the calculated mass in oxidized state.

3 Long-term stability and offered formats

Figure 4

A



B

Miltenyi Biotec products	MACS GMP Nuclease A - *small*	MACS GMP Nuclease A - *medium*	MACS GMP Nuclease A - *large*
Units [total]	100,000 U	1,000,000 U	4,000,000 U (4x 1,000,000)
LV process volume*	5 L	50 L	200 L
vial format	2 R glass vial, single use	10 R glass vial, single use	10 R glass vial, single use
concentration [U/µL]	min. 250	min. 250	min. 250

*according to our internal LV production pipeline (Miltenyi Biomedicine)

A) R&D feasibility study on long term stability shows almost no decrease in activity for > 4 years at storage of functional sample at -20 ° C.
B) MACS GMP Nuclease A is available in three vial formats ranging from *small* for feasibility studies in approx. 5 L scale up to *medium* and *large* vial formats fitting into upscale (50 L) and pilot lot productions for up to 200 L.

Functionality study

1 Transducing titer determination shows reliable performance

Figure 5

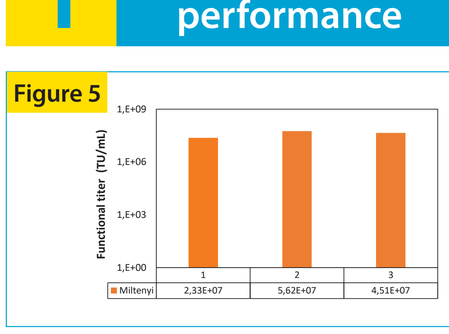


Figure 5: Functional titers of lentiviral vector productions (n=3) in 4 L scale, measured by GagDuplex assay [TU/mL].

2 Total clearance of residual DNA

Figure 6

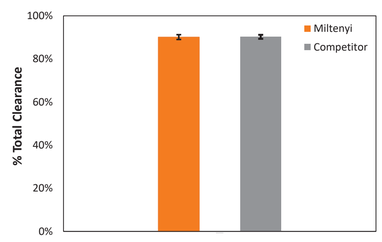


Figure 6: Percentage residual DNA below 200 bp in LVV productions in comparison Miltenyi vs. competitor nuclease (n=3). MACS GMP Nuclease A drastically decreased (> 90 %) amount of residual DNA and reduced the size of residual DNA to below the size of a functional gene (approx. 200 bp) determined by fragment analyzer.

3 Impurities clearance during vector purification

Figure 7

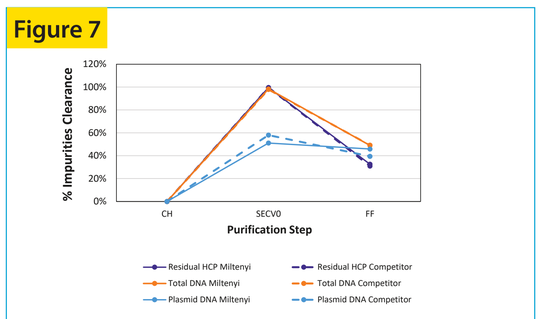


Figure 7: Overview of process steps in LVV production with stepwise clearance. Graphic starts with CH (clarified harvest), followed by SEC (Size Exclusion Chromatography) and lastly FF (Final Fill). Analyzed are HCP (Host Cell Protein), total DNA and plasmid DNA by PicoGreen assay and fragment analyzer.

Figure 8

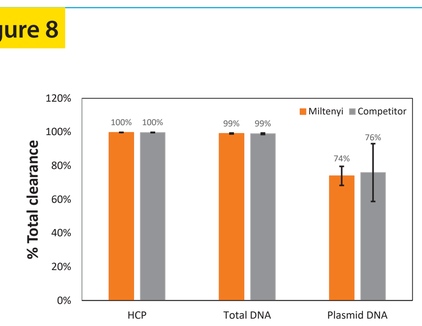


Figure 8: Total clearance in percentage of MACS GMP Nuclease A in comparison to a competitor product in aspects of HCP, total DNA and plasmid DNA.

Conclusion

The aim of this study was to determine whether the set specifications of MACS GMP Nuclease A are sufficient to reach the goal of providing a reagent equivalent or superior to a *state-of-the-art* product for usage in lentiviral production at Miltenyi Biotec. To determine this, FF and SEC V0 samples were analysed for physical titer, functional titer, residual DNA size, HCP, Picogreen data and residual plasmid DNA. In addition, the cumulative clearance of HCP, residual DNA and residual plasmid DNA was compared between the two Nuclease A types.

From these results it can be concluded that the *state-of-the-art* product and MACS GMP Nuclease A are comparable in performance and application of MACS GMP Nuclease A should be considered. Furthermore, it should be emphasized that Miltenyi Biotec with its affiliates Bioindustry and Biomedicine has long standing experience in cell and gene therapy market and has thus already proven its efficiency in the field by developing by its own (e.g. Zamtocabtagene autoleucel) or supporting as contract manufacturer (e.g. TECELRA®) several clinical trial studies employing lentiviral vector productions.

Figure 9

Miltenyi Bioindustry Contract Manufacturing

MACS GMP Nuclease A successfully implemented in our processes