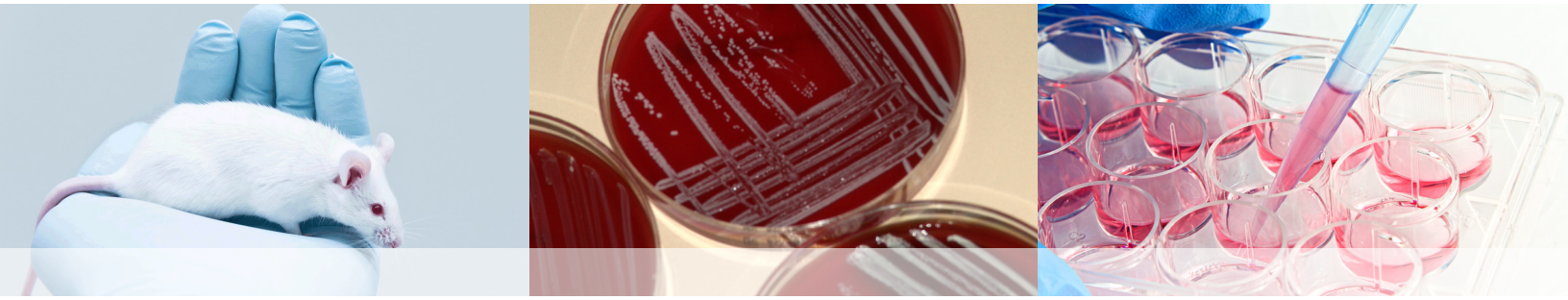
MACHEREY-NAGEL

NucleoSpin® 96 DNA RapidLyse

Automated purification of DNA from various tissues using the TECAN Freedom EVO® 150



Introduction

DNA extraction from various tissue samples comes along with matrix specific challenges. Therefore, genomic DNA purification from tissues like mouse tails, mammalian organs, or even eukaryotic cells can be an elaborate and time consuming task. An efficient sample lysis and DNA release is essential for subsequent downstream molecular applications, utilized by many research laboratories. MACHEREY-NAGEL designed the silica membrane based NucleoSpin® 96 DNA RapidLyse kit with a unique buffer chemistry to enable a shortened cost efficient purification workflow.

The external sample lysis can be performed within 15–60 min depending on sample material. High quality DNA is subsequently extracted on the Freedom EVO® 150 and can directly be used as a template for PCR, NGS, blotting, or various other enzymatic reactions. This silica membrane based kit can be successfully used with either centrifugation or standard vacuum processing in manual or automated manner.

This Application Note describes the automated processing of the NucleoSpin® 96 DNA RapidLyse kit from MACHEREY-NAGEL on the liquid handling Freedom EVO® 150 platform using the Te-VacS™ for vacuum processing including the Vacuum Block Type C and Spacer for Te-VacS™ Type No. 1 and No. 6. The novel optimized protocol allows the processing of variable sample numbers. The processing of 96 samples takes approximately 90 minutes excluding external sample lysis.

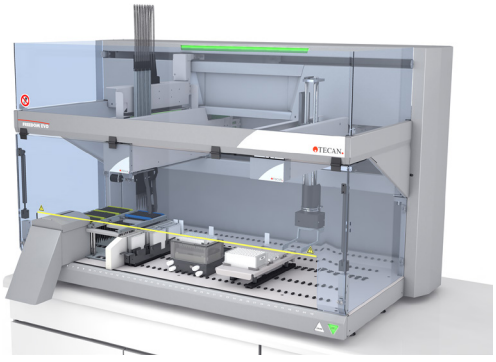
Product at a glance

NucleoSpin® 96 DNA RapidLyse	
Technology	Silica membrane technology
Sample material	≤ 30 mg tissue, < 10^6 cells
Typical yield	Up to 4 µg DNA/mg tissue or 10^6 cells
Preparation time	Approx. 90 min for 96 samples (excluding lysis). Variable sample number in multiples of 8 (8–96)
Elution volume	100 µL
Theoretical binding capacity	40 µg

Freedom EVO® 150	
Capacity	8–96 samples per batch (in increments / multiples of 8)
Batch processing time	~1.5 h for 96 samples using NucleoSpin® DNA RapidLyse (excluding lysis)
Special features	TouchTools™ software for intuitive touch screen guided operation, reduces training needs, Integration of an Infinite® F or M NANO+ reader (Configurations of the Infinite 200 PRO family), in combination with the Freedom EVOware® Normalization Wizard, allowing for automated quantification and normalization

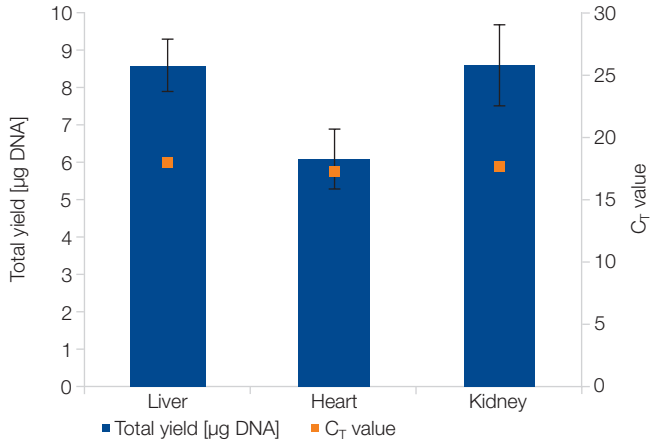
Material and methods

The optimized protocol is programmed to process up to 96 samples in parallel (multiples of 8) and developed for the Freedom EVO® platform. DNA was isolated from various mouse tissue samples (30 mg each) and were lysed in maximal one hour agitated incubation at 56 °C. The highly efficient DNA release is enabled by a thoroughly designed lysing setup with optimized parameters that comprise the special Lysis Buffer RLY in combination with Liquid Proteinase K. Incubation over night or for several hours is not necessary. Nucleic acids are reversibly bound to the silica membrane during the binding step. Contaminants, such as salts or lipids, are then removed from the silica membrane by three washing steps using Washing Buffer RLW, while nucleic acids stick to the silica membrane. Highly pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline Elution Buffer RLE.



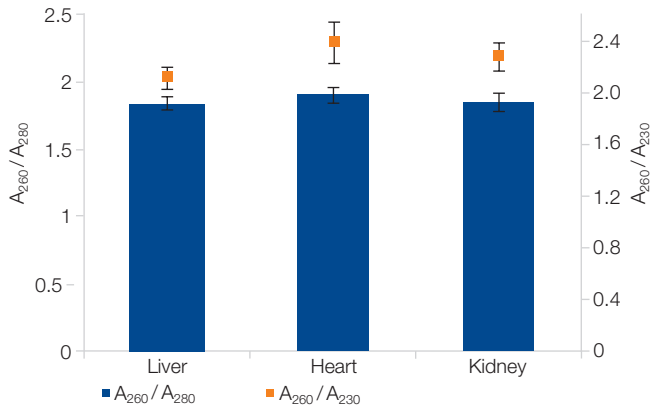
Example configuration on a Freedom EVO® 150

Application data



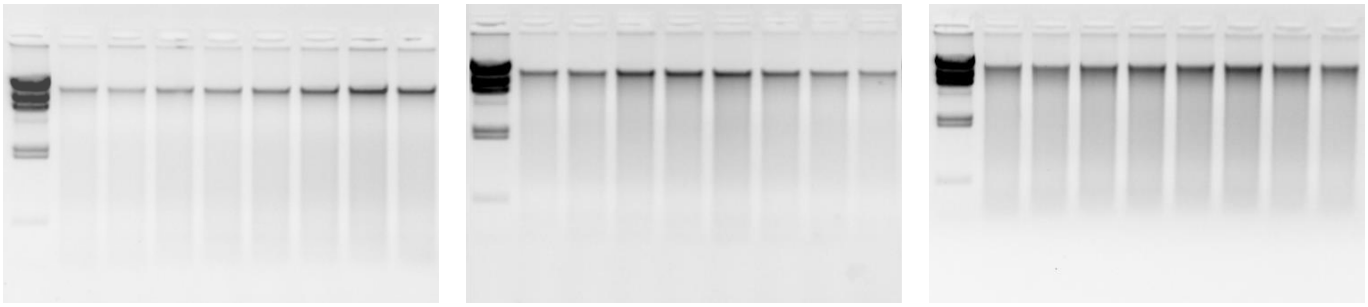
Automated isolation of genomic DNA from mouse organs

DNA was isolated from various mouse tissue samples (n= 8, 30 mg each) using the NucleoSpin® 96 DNA RapidLyse kit on a Freedom EVO® 150 platform. The total yield was determined by UV spectrometry (dark blue bars). A subsequent qPCR analysis (orange squares) was performed with a Taqman® Probe for a GAPDH amplicon using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.



Purity of isolated genomic DNA from mouse organs

DNA was isolated from various mouse tissue samples (n = 8, 30 mg each) using the NucleoSpin® 96 DNA RapidLyse kit on a Freedom EVO® 150 platform. The purity was determined by UV spectrometry, resulting in an average A_{260}/A_{280} value for liver tissue of 1.84 ± 0.05; for kidney tissue of 1.85 ± 0.07; for heart tissue of 1.9 ± 0.06 (dark blue bars). The average A_{260}/A_{230} value for liver tissue = 2.11 ± 0.09 for kidney tissue = 2.27 ± 0.11; for heart tissue = 2.38 ± 0.16 (orange squares).



Integrity of isolated DNA from mouse organs

The integrity of the isolated nucleic acids from mouse organ samples was analyzed by gel electrophoresis (2 µL per eluate; 1 % TAE gel; M: Lambda DNA/Hind III – Thermo Scientific)

Automate your gDNA extraction on the Freedom EVO® 150 platform

MACHEREY-NAGEL and TECAN deliver an automated solution for your high throughput DNA extraction from various tissue samples We adapted the NucleoSpin® 96 DNA RapidLyse kit on the Freedom EVO® 150 to speed up your nucleic acid purification workflow.

- Reliable performance and excellent yields using NucleoSpin® 96 DNA RapidLyse on the Freedom EVO® 150
- Optimized protocol allowing flexible sample numbers in multiples of 8 samples
- Fast processing of 96 samples within 90 minutes (excluding lysis)

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® 96 DNA RapidLyse	Kit based on silica membrane technology for fast isolation of genomic DNA from a variety of sample materials in 96-well format	1 x 96 4 x 96	740110.1 740110.4
Square well Block	Square-well Block with 2.5 mL square wells suitable for lysis or elution	4 24	740481 740481.24