

NucleoSpin[®] 96 Plasmid / ASSIST PLUS

Automated purification of plasmid DNA using INTEGRA's ASSIST PLUS pipetting robot

Introduction

Isolation of plasmid DNA from bacterial cultures is an essential, yet time-consuming workflow in many molecular biology laboratories. With the NucleoSpin[®] 96 Plasmid Kit, MACHEREY-NAGEL offers an established method for the parallel extraction and purification of high-quality plasmid DNA in a 96-well format for use in a manual or automated fashion. The silica-based technology yields plasmid DNA of high quality for subsequent applications, such as cloning or sequencing.

This application note presents the first implementation of the MN NucleoSpin[®] 96 Plasmid Kit on the ASSIST PLUS pipetting robot from INTEGRA Biosciences. The combination of these two technologies results in a reduction of pipetting errors as well as superior reproducibility through workflow consistency, thereby enabling a fast and convenient way to achieve precise results. The use of the INTEGRA's VIAFLO electronic pipette in combination with GripTips ensures accurate and error-free pipetting to complete a 96-sample run in less than 1 h.

Product at a glance

NucleoSpin [®] 96 Plasmid	
Technology	Silica membrane technology
Sample material	Up to 2.5 mL bacterial culture (<i>E. coli</i> , high-copy plasmid)
Preparation time	Approx. 50 min
Typical yield	4 – 6 µg/mL <i>E. coli</i> culture
Elution volume	75–150 µL
Theoretical Binding capacity	20 µg
ASSIST PLUS	
Technology	Air displacement
Sample volume	Up to 2.5 mL bacterial culture (<i>E. coli</i> , high-copy plasmid)
Capacity	1x 96 samples
Size (WxDxH) / weight	75 x 38 x 63 cm / 25 kg



Materials and methods

pGEM[®]-T Easy variants with insert sizes of 645, 982 and 1519 bp as well as pcDNA3.1 were produced in shaking flasks containing recombinant *E. coli* cultures. Aliquots of 2 mL, 1.3 mL and 0.65 mL were harvested in a culture plate. After resuspension and lysis of the cells, the samples were neutralized by addition of Buffer A3. Cellular debris and chromosomal DNA were removed using the NucleoSpin[®] 96 Plasmid Filter Plate. Plasmid DNA was subsequently bound to the silica membrane of the NucleoSpin[®] 96 Plasmid Binding Plate.

Three washing steps ensure the removal of salts and impurities before the highly pure plasmid DNA is eluted under low ionic strength conditions with Buffer AE.

All steps of the plasmid purification procedure (pellet resuspension, lysis, neutralization and clearance, plasmid DNA binding and washing steps, as well as elution) were performed on the ASSIST PLUS in a semi-automated manner.

Application data

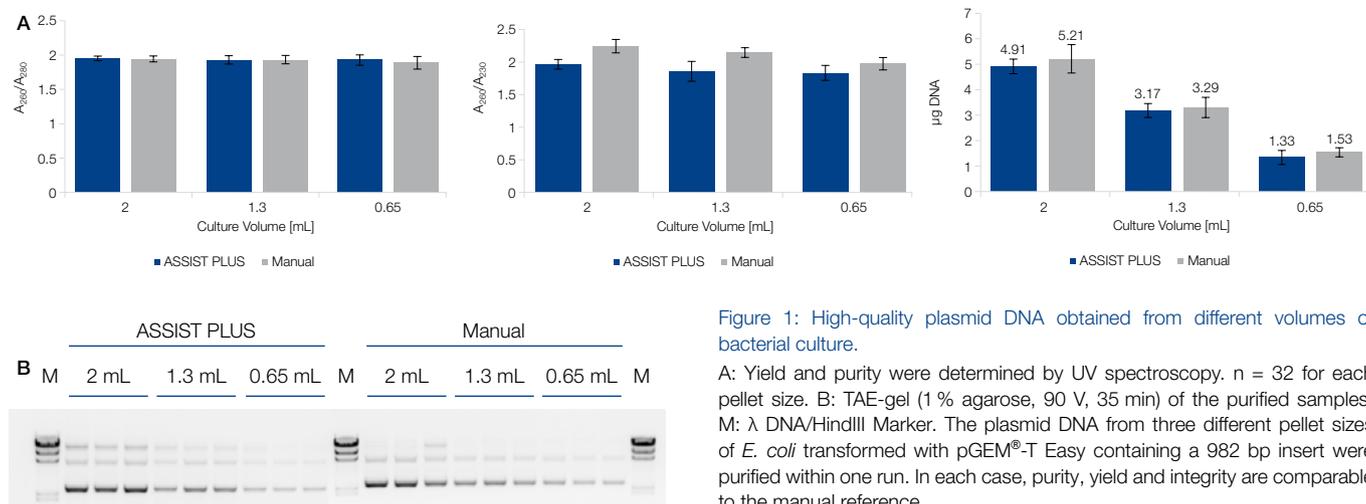


Figure 1: High-quality plasmid DNA obtained from different volumes of bacterial culture.

A: Yield and purity were determined by UV spectroscopy. $n = 32$ for each pellet size. B: TAE-gel (1% agarose, 90 V, 35 min) of the purified samples. M: λ DNA/HindIII Marker. The plasmid DNA from three different pellet sizes of *E. coli* transformed with pGEM[®]-T Easy containing a 982 bp insert were purified within one run. In each case, purity, yield and integrity are comparable to the manual reference.

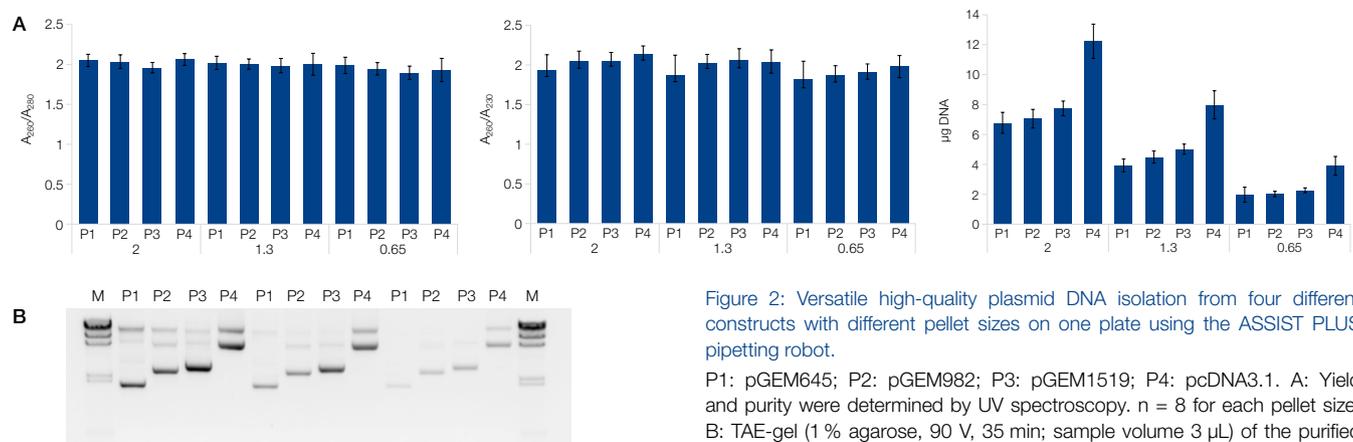


Figure 2: Versatile high-quality plasmid DNA isolation from four different constructs with different pellet sizes on one plate using the ASSIST PLUS pipetting robot.

P1: pGEM645; P2: pGEM982; P3: pGEM1519; P4: pcDNA3.1. A: Yield and purity were determined by UV spectroscopy. $n = 8$ for each pellet size. B: TAE-gel (1% agarose, 90 V, 35 min; sample volume 3 μL) of the purified samples. M: λ DNA/HindIII Marker

Automate your plasmid DNA extraction

MACHEREY-NAGEL and INTEGRA deliver a sophisticated, semi-automated solution for a fast, reliable and convenient plasmid preparation experience.

- Reproducible performance and excellent yields using NucleoSpin[®] 96 Plasmid on the INTEGRA ASSIST PLUS
- Compact semi-automated processing of 96 samples in less than 1 h

Ordering information

Product	Specifications	Pack of	REF
ASSIST PLUS pipetting robot	Includes base unit, 3 position universal deck, tip waste bin with frame and reflector, pipette charging cable, power cord and USB cable		INTEGRA Biosciences 4505
VIAFLO	12 channel electronic pipette 50–1250 μL		INTEGRA Biosciences 4634
1250 μL Tips	Sterile, Filter GripTips	5 x 96 tips	INTEGRA Biosciences 6445
NucleoSpin [®] 96 Plasmid	Kit based on silica-membrane technology for the isolation of plasmid DNA from bacterial cultures in 96-well format	1 x 96	740625.1
		4 x 96	740625.4
		96 x 96	740625.24
NucleoVac 96 Vacuum Manifold	Vacuum manifold consisting of manifold base and lid, a spacer set, and two waste containers	1	740681

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