Promega

Product Application

In collaboration with: dynamicdevices

Rapid Purification of Cell-Free DNA from 10ml Plasma on the Dynamic Devices Lynx

Purify cell-free DNA from 10ml plasma with the Maxwell® HT Rapid ccfDNA Kit on the Dynamic Devices Lynx robotic liquid handler.

Kit: Maxwell® HT Rapid ccfDNA Kit (Cat.# A6080)

Analyses: Dye-based quantitation, qPCR, ScreenTape Analysis

Sample Type(s): Plasma collected in Streck Cell-Free DNA BCT®

Input: 10ml

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® HT Rapid ccfDNA Kit (Cat.# A6080)
- Maxwell® HT Rapid ccfDNA Resin (RCFD) (Cat.# MC1561)
- Proteinase K Solution (Cat.# MC5005)
- 2 – Maxwell® HT Rapid ccfDNA Elution Buffer (RCFD) (Cat.# MC1591)
- Dynamic Devices Lynx LM1200 Robotic Liquid Handler
    - 24ST Pipetting Tool
    - 96ST Pipetting Tool
    - 6 – iMagZ Magnet Elevator
    - Passive Waste Block
    - Source Tube Holders
- 4 – MagPlate 24 (Alpaqua, Cat.# A000270)
- 2 – Magnum FLX® 96 Magnet with Solid-Core™ Technology (Alpaqua, Cat.# A000400)* *Replacement of the Magnum FLX® 96 Magnet with the 96S Super Magnet (Alpaqua, Cat.# A001322) may allow a reduced elution volume of 50µl (versus 75µl as described here)

Plastic Consumables Required per 24/96 Samples:

- DDX 24 5mL Tips Filtered (Dynamic Devices, Cat.# DDX-24-5000F-1) [3/6 boxes]
- DDX 1250µL Tips Filtered (Dynamic Devices, Cat.# DDX-96-1250F-1) [5/5 boxes]
- 300ml Reagent Reservoir with Reservoir Base (Integra, Cat.# 6309) [5/5 reservoirs]
- 96-Well No Bottom Microplate (Greiner Bio-One, Cat.# 655000) [1/1 plate]
- 24 Deepwell Plate – Pureprep 24 (MolGen, Cat.# MG24020050-01) [1/4 plates]
- Nunc™ 96-Well Polypropylene DeepWell™ Sample Processing and Storage Plates, 2000µl (Thermo Scientific™, Cat. #278743) [2/2 plates]
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/clear (Bio-Rad, Cat. #HSP9601) [1/1 plate]

Lynx Method: Lynx_10ml_RapidccfDNA.met

Method Timing: 2.5 hours for 96 samples

Protocol:

1. Fill the 300ml reagent reservoirs with the volumes indicated in Table 1.

Reagent	Volume per Sample	Volume Required for 96 Samples‡	Volume Required for 24 Samples‡
Binding Buffer (RCFD)	1.31ml	142ml	48ml
Maxwell® Resin (RCFD)	0.94ml (Initial Bind) 0.25ml (Second Bind)	131ml	45ml
Wash 1 (RCFD1) with Proteinase K (ProK)†	1ml Wash 1 (RCFD1) 25µl ProK	112ml Wash 1 + 2.8ml ProK	40ml Wash 1 + 1ml ProK
Wash 2 (RCFD2)	0.5ml	64ml	28ml
Elution Buffer (RCFD)	1ml (Intermediate Elution) 75µl (Final Elution)	120ml	42ml

‡Indicated volumes include required dead volume for the reagent troughs.

†To be prepared as a master mix of Wash 1 (RCFD1) and ProK before loading on the instrument.

2. Set up the instrument deck according to Figure 1.

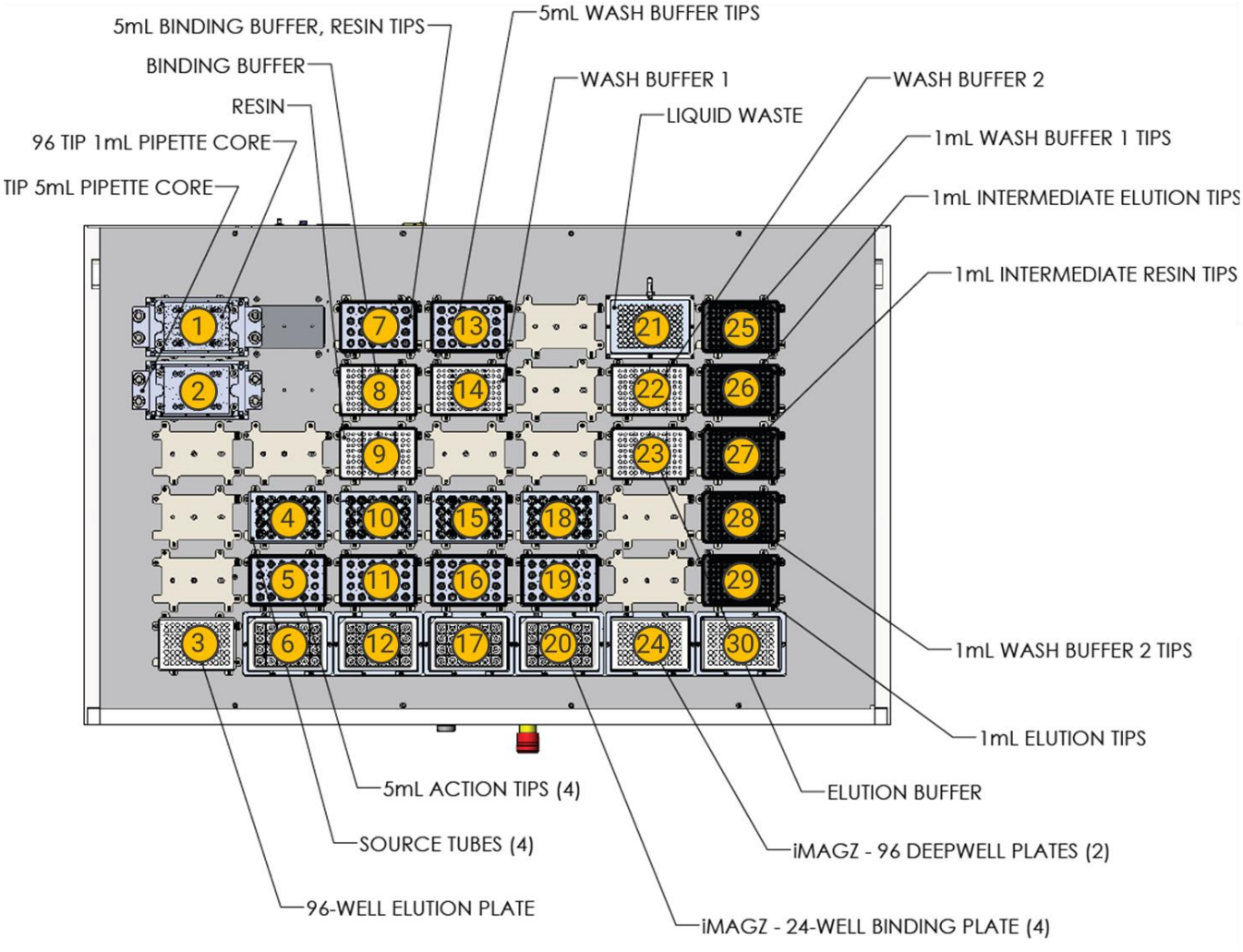

Figure 1. Deck setup for processing 4ml plasma samples with the Maxwell® HT Rapid ccfDNA Kit on the Lynx LM1200 Robotic Liquid Handler. Reagent and consumable deck locations are summarized in the table below.

	Hardware/Consumable	Position(s)
Instrument Hardware	96 Tip 1ml Pipette Core	1
	24 Tip 5ml Pipette Core	2
	Passive Waste Block with No-Bottom Microplate	21
Reagents	Binding Buffer (RCFD)	8
	Maxwell® Resin (RCFD)	9
	Wash Buffer (RCFD1) with Proteinase K	14
	Wash Buffer (RCFD2)	22
	Elution Buffer (RCFD)	23
Tips	5ml Tips	5, 7, 11, 13, 16, 19
	1ml Tips	25, 26, 27, 28, 29
Plates	24 Deepwell Plate	6, 12, 17, 20

	2ml Nunc™ 96-DeepWell™ Plate	24, 30
	Hard-Shell® 96-Well PCR Plate	3
Source Tubes	Sample Input Tubes	4, 10, 15, 18

Method Description:

Initial Cell-free DNA Binding:

- 10ml sample is transferred from the source tubes to the 24-well processing plate.
- 1.31ml Binding Buffer (RCFD) and 940µl well-resuspended Maxwell® Resin (RCFD) are added to the sample.
- The sample is bound for a total of 24 minutes, with tip mixing with 5ml tips at t = 0 and again at 6-minute intervals.
- The iMagZ raises the magnets to the active position, and the resin is allowed to collect for 10 minutes.
- The supernatant is removed from the sample.

Wash 1 and Sample Transfer to 96-well Processing Plate:

- The iMagZ lowers the magnets to the inactive position.
- 1ml Wash Buffer 1 (RCFD1) with ProK is added to each well, and the resin is resuspended using a rigorous tip mixing protocol with the 5ml tips.
- The resuspended resin is transferred to the first 96-well Processing Plate.
- After all resin is transferred, the resin is resuspended using a rigorous tip mixing protocol with the 1ml tips.
- The iMagZ raises the magnets to the active position, and the resin is allowed to collect for 2 minutes.
- The supernatant is removed from the sample.

Intermediate Elution and Sample Transfer to the Second 96-well Processing Plate:

- The iMagZ lowers the magnets to the inactive position.
- 1ml Elution Buffer (RCFD) is added to each well, and the resin is resuspended using a rigorous tip mixing protocol with the 1ml tips.
- The intermediate elution is incubated for a total of 13 minutes with interval tip mixing.
- The iMagZ raises the magnets to the active position, and the resin is allowed to collect for 2 minutes.
- The supernatant is transferred to the second 96-well Processing Plate.

Second Cell-free DNA Binding:

- 250µl well-resuspended Maxwell® Resin (RCFD) is added to each well, and the resin is resuspended using a rigorous tip mixing protocol with the 1ml tips.
- The sample is bound for a total of 13 minutes with interval tip mixing.
- The iMagZ raises the magnets to the active position, and the resin is allowed to collect for 2 minutes.
- The supernatant is removed from the sample.

Wash 2:

- The iMagZ lowers the magnets to the inactive position.
- 500µl Wash Buffer 2 (RCFD2) is added to each well, and the resin is resuspended using a rigorous tip mixing protocol with the 1ml tips.
- The iMagZ raises the magnets to the active position, and the resin is allowed to collect for 1 minute.

- The supernatant is removed from the sample, ensuring that the Wash Buffer 2 (RCFD2) is removed completely.

Elution:

- The iMagZ lowers the magnets to the inactive position.
- 75µl Elution Buffer (RCFD) is added to each well, and the resin is resuspended using a rigorous tip mixing protocol with the 1ml tips.
- The iMagZ raises the magnets to the active position, and the resin is allowed to collect for 1 minute.
- The eluted DNA is transferred to the final elution plate.

Results:

The protocol described above was used to purify cell-free DNA from 10ml of plasma fractionated from whole blood collected in Streck Cell-Free DNA BCT® tubes. Samples from 3 individuals were purified in triplicate. Cell-free DNA yield and quality was assessed as described in Figure 2. The size profile based on electrophoresis and yield were within the expected limits for cell-free DNA.

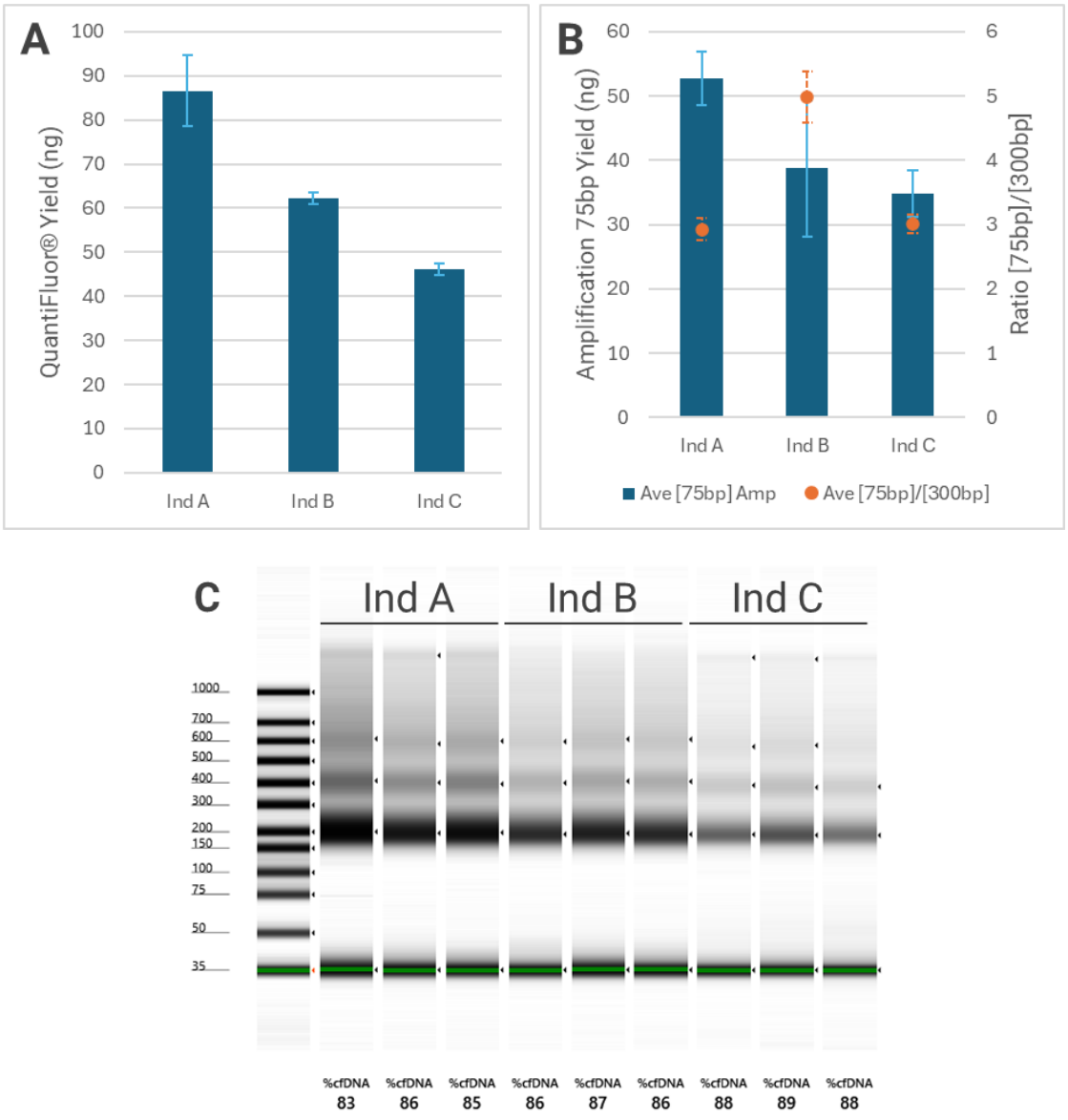

Figure 2. Quantification and size analysis of cell-free DNA purified using the Maxwell® HT ccfDNA Kit on the Lynx LM1200 Robotic Liquid Handler. Blood from three individuals was collected in Streck Cell-Free DNA BCT® tubes. Plasma was isolated by double-spin centrifugation, and 10ml was processed in triplicate. (A) Yield quantified by QuantiFluor® ONE dsDNA System (mean ± SD). (B) Yield calculated from ProNex® DNA QC Assay using a 75bp amplicon (mean ± SD). The [75bp]/[300bp] amplicon ratio indicates genomic DNA contamination; higher ratios reflect less contamination. (C) DNA size profile analyzed using Agilent TapeStation Cell-Free DNA ScreenTape.