



Automated Genomic DNA Isolation with Invitex's Blood Mini HTS 96 Kit Using Aurora's VERSA™ Mini NAP-Vacuum Manifold Workstation

Cat #: 71313203

1 Objectives

- To automate genomic DNA isolation from whole blood using Invitex's Invisorb® Blood Mini HTS 96 Kit on the VERSA™ Mini NAP-Vacuum Manifold Workstation
- To validate the VERSA™ Mini robotic liquid handling workstation equipped with Vacuum manifold
 - To determine DNA purity and yield
 - To determine quality of gDNA for downstream realtime PCR based applications
 - To test for cross-contamination among samples

2 Introduction

The VERSA™ Mini robotic liquid handling workstation in combination of a genomic DNA (gDNA) isolation kit, such as the Invisorb® Blood Mini HTS 96 Kit, provides a convenient tool for a fast and efficient walk-away automated isolation and purification of genomic DNA (gDNA) from as little as 200 µl of fresh or frozen whole blood on a 96 well microplate. Automated sample preparation enables efficient, standardized purification of nucleic acids. The purified DNA can be used for routine *in vitro* diagnostic analysis. The kit is designed for simultaneous processing of 96 samples. All processing steps are performed by the VERSA™ Mini robotic workstation equipped with a vacuum. The whole blood sample is lysed in an optimized lysis buffer and proteins are degraded during the lysis with Proteinase K direct on the robotic platform. The DNA binds to filter membrane, followed by washing steps and the final elution.

Manual handling of potentially infectious samples is minimized, ensuring safety to the user and reliable processing of samples. The streamlined procedure provides robust walk-away purification of nucleic acids with minimal hands-on time. The purified genomic DNA is of high quality and can be used for a broad panel of subsequent downstream applications, such as PCR, real-time PCR, restriction enzyme digestion, hybridization, SNP analysis, and genotyping. Automated DNA purification provides high levels of reliability and standardization and helps to eliminate variation caused by handling errors.

3 Materials and Methods

1. **Deck set up:** The Invisorb® Blood Mini HTS 96 Kit (Invitex, Germany) was used to extract genomic DNA (gDNA) from 200 µL of human whole blood samples (n = 8) on the VERSA™ Mini robotic workstation equipped with a vacuum system. DEPC treated water sample (n = 8) was used as a negative control. A reagent holder block and up to three microplates and three pipette tip boxes were placed on the VERSA™ Mini deck (Figure 1). Samples were positioned alternatively in a

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checkerboard pattern to screen for cross-contamination among samples (Figure 2). The eluted DNA was diluted 6-fold for further analysis.

- Liquid handling:** The samples were processed following the protocol described in the product insert of the kit after placing the reagents in the cooling block etc. On the wash, and elution module, the vacuum of 9 psi was applied by to effect the wash, and elution steps. The entire process from sample lysis to elution was carried at room temperature (22°C+/- 1°C).
- Quality parameters:** To determine the purity of the isolated DNA, absorbance readings at 260, and 280 nm were taken and the ratios of A260/280 were calculated. The yield of DNA was determined by NanoDrop spectrophotometer. The quality of the gDNA was also verified by amplification of GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), a housekeeping gene to determine its suitability for realtime PCR based downstream applications.

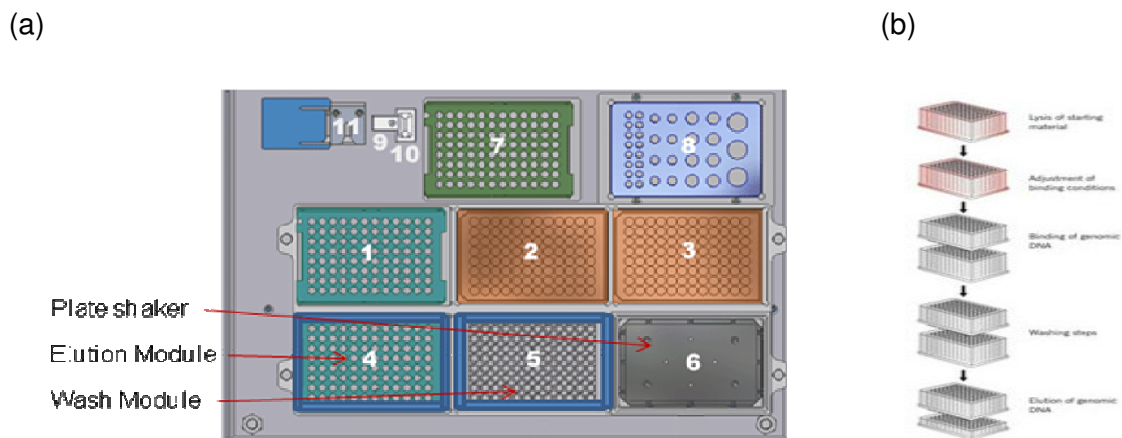


Figure 1: (a) VERSA™ Mini deck layout (Tip boxes: 20, 200, and 100 µL on deck position 1, 2, and 7, respectively. Sample plate on deck position 3; lysis plate on shaker at deck position 6; Wash module at deck position 5, and elution module on deck position 4. Liquid waste disposal at deck position 11. Thermolabile reagents at deck position 8). (b) Process overview.

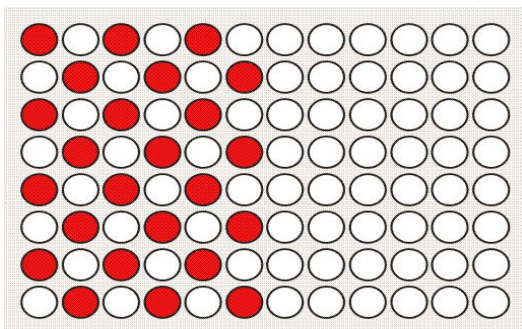


Figure 2: Illustration of sample placement configuration on a 96-well microplate to screen for cross-contamination (red circle: blood; white circle: DEPC water)

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4 Results

The Invitek's Invisorb[®] Blood Mini HTS 96 Kit **has been successfully validated** on the VERSA[™] Mini robotic liquid handling workstation.

4.1 Real-time PCR of GAPDH gene for cross-contamination

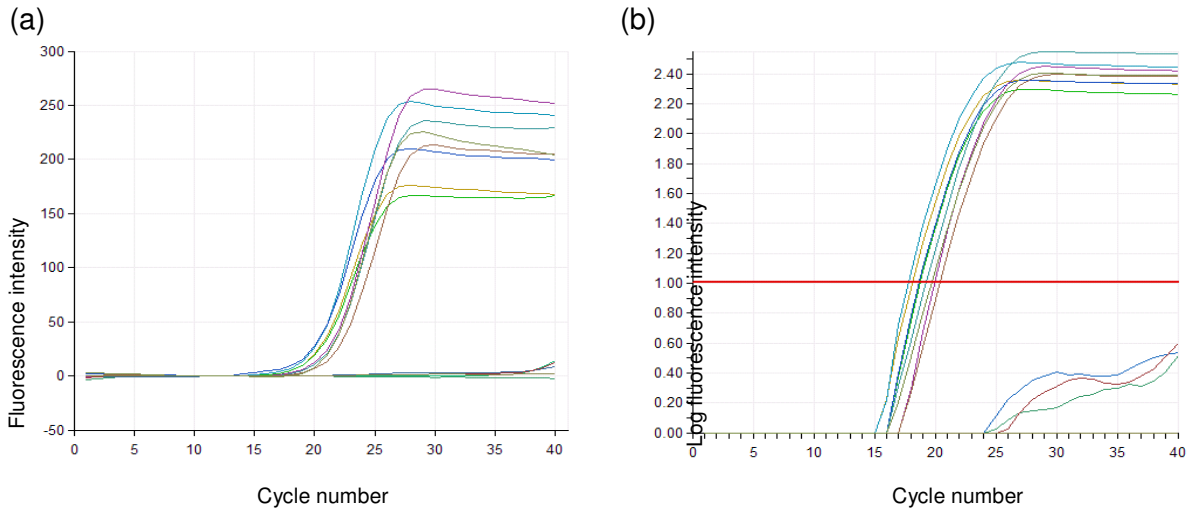


Figure 3: Amplification curves of baseline (a) and threshold value (b) setup. (n=16, 8 samples and 8 blank). The ct values for DNA containing samples, and no-amplification from water samples containing no DNA.

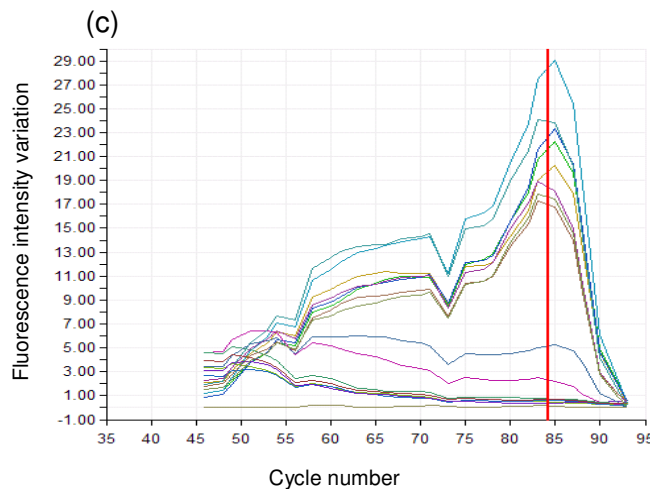


Figure 4: The DNA melting curve of the resulting product validating the integrity of PCR reactions, and the amplicons.

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4.2 Reproducibility and no PCR inhibitors: The data on the 260/280 on the isolated DNA sample having a CV of 2.9% show significant degree of reproducibility on DNA purity, and absence of any PCR inhibitors in the DNA preparations (Table 1).

4.3 Purity and yield of the isolated gDNA

The yield of the DNA having a CV of 20% is close to the manual process. The DNA preparations being significantly pure provide more than sufficient amount of DNA for downstream applications (Table 1).

Table 1. Purity and yields of the eluted blood gDNA after a 6-fold dilution.

	Sample number							
	1	2	3	4	5	6	7	8
A260	0.097	0.115	0.105	0.061	0.075	0.094	0.082	0.072
A280	0.056	0.067	0.057	0.034	0.042	0.052	0.044	0.039
A260/280	1.73	1.72	1.84	1.79	1.79	1.81	1.86	1.85
Concentration ($\mu\text{g}/\text{mL}$)	29.1	34.5	31.5	18.3	22.5	28.2	24.6	21.6
Yield ($\mu\text{g}/200 \mu\text{L}$ blood)	5.82	6.9	6.3	3.66	4.5	5.64	4.92	4.32

5 Conclusion

Blood genomic DNA (gDNA) isolation using the Invisorb® Blood Mini HTS 96 Kit was successfully validated on the VERSA™ Mini NAP/PCR automated liquid handling workstation. The vacuum system of the automated workstation effectively eliminated cross-contamination among sample wells. The isolated gDNA are of high purity with a A260/280 ratio of 1.72 - 1.86 and of considerable yield of 3.7 - 6.9 $\mu\text{g}/200 \mu\text{L}$ blood.

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