MBIN020 Insta NX® Forensic Multi-Sample DNA Purification Kit

Kit Contents

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents provided</th>
<th>12 Preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS0399</td>
<td>Multi-Sample DNA Extraction Cartridges</td>
<td>12 nos.</td>
</tr>
<tr>
<td>DS0337</td>
<td>1 ml tip</td>
<td>12 nos.</td>
</tr>
<tr>
<td>PW1139</td>
<td>Collection Tubes, Polypropylene (2.0 ml)</td>
<td>30 nos.</td>
</tr>
<tr>
<td>MB086</td>
<td>Proteinase K</td>
<td>10 mg</td>
</tr>
<tr>
<td>DS0015</td>
<td>Lysis Solution (AL)</td>
<td>5 ml</td>
</tr>
<tr>
<td>DS0040</td>
<td>Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]</td>
<td>350 µl</td>
</tr>
<tr>
<td>DBCA021</td>
<td>Columns for Insta NX</td>
<td>12 nos.</td>
</tr>
</tbody>
</table>

Intended Use

Recommended for isolation of RNA from human blood/animal blood, plasma, serum and other samples such as cells, tissues, stool sample and body fluids.

Introduction

This kit provides a fast and easy method to purify total DNA from various forensic samples listed above for downstream applications such as PCR, Southern blotting technique etc. The DNA purification procedure using the column comprises of three steps viz. adsorption of DNA to the Super- S membrane, removal of residual contaminants and elution of pure DNA. The Super- S columns have a high binding capacity and high quality genomic DNA is obtained from various species.

Insta NX® Forensic Multi-Sample DNA Purification Kit

This kit simplifies isolation of DNA from fresh or old (more than 24 hours) samples with vacuum based innovative Super-S column technology. The Super- S columns contains specially developed membranes for optimal binding of genomic DNA. After the initial binding of DNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality DNA is finally eluted in the Elution Buffer.

The salient features of this kit are as follows:

a. Cross- Contamination free
b. 12 samples can be processed at a time.
c. Prefilled reagent cartridge
d. High purity, high yield, as good as manual.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. An elution with 50 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reactions. The eluted DNA ranges in size upto 20-30 kb, and is suitable for direct use in PCR, restriction digestion and Southern blotting applications.
Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer (ET) to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz micro cuvette.

Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The $A_{260} - A_{320} / A_{280} - A_{320}$ ratio should be 1.6-1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by Insta NX® Forensic Multi-Sample DNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of DNA sample ($\mu g/ml$) = $50 \times A_{260} \times$ dilution factor.

Storage

Store the Insta NX® Forensic Multi-Sample DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 4 months.

Materials needed but not provided

- Insta NX® (LA1056)
- Heating Block at 55°C
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- 1M DTT (Dithiothreitol) solution (MB070)
- Vortex Mixer
- Micropipettes (LA617, LA613) and Tips (LA974)
- Molecular Biology Grade Water (ML024)

General Preparation Instructions

- **IMPORTANT:** Please go through the instruction manual before starting the experiment.
- **Reconstitute Proteinase K (MB086) (PROVIDED)**
  
The Insta NX® Forensic Multi-Sample DNA Purification Kit contains Proteinase K. Intensive research has shown that it is the optimal enzyme for use with the Lysis Solution provided in the kit. It is completely free of DNase and RNase activity. Proteinase K is the enzyme of choice for use with an SDS containing Lysis Solution. The specific activity of Proteinase K is 33.5 units/mg dry weight. Resuspend the Proteinase K (MB086) powder in Molecular Biology Grade Water (ML024) to obtain a 20 mg/ml stock solution.

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Proteinase K</th>
<th>Molecular Biology Grade Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>10 mg</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

- **Reconstitute DTT (DL-Dithiothreitol) (MB070) (NOT PROVIDED)**
  Resuspend the DTT (DL-Dithiothreitol) powder in Molecular Biology Grade Water (Product code: ML024) to obtain a 1M stock solution.

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>DTT (DL-Dithiothreitol)</th>
<th>Molecular Biology Grade Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>18.51 mg</td>
<td>0.12 ml</td>
</tr>
</tbody>
</table>

The product is stable at 2-8°C; upon reconstitution store at -20°C in dark (wrapped in an aluminum foil), as mentioned in storage instructions.
NOTE: The 1M DTT solution must be added directly to each sample preparation every time.

Specimen Handling and Collection
Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage.

Collect cells, tissues, Serum, Plasma, stool sample and body fluids in a clean sterile container and store the samples at 2-8°C for short term storage or -20°C for long term storage. Repeated freeze-thaw of samples should be avoided.

Ensure that the blood/tissue/cells/stool/bacterial culture/yeast Culture, Serum, Plasma and body fluids sample is at room temperature (15-25°C) before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen
Clinical samples: Whole blood, tissues, stool and body fluids.

Procedure (For Non-Barcode Mode)
1. Turn on Insta NX® instrument. Allow the instrument to initialize.
2. Once the instrument is ready, select “Login” on the instrument screen.

![Fig. 1](image1)

3. Enter the Password to login.

![Fig. 2](image2)
4. On the Home screen, select “Start Purification”

![Home screen with Start Purification option](image)

Fig. 3

5. On the Product Type screen, select the type of purification to be carried out.

![Product Type screen with DNA, RNA, and Virus options](image)

Fig. 4

6. On the Setting screen
   a. Select Elution volume: 50/100/150 or 200µl
   b. Select Kit Name (type of extraction procedure to be performed).

![Setting screen with Elution Vol. 200 µl and Kit Name MBIN020 Insta NX® Forensic Multi-Sample](image)

Fig. 5

7. Lift open the front door and take out the 12-in-1 rack for preparation.
   **NOTE:** You can follow Step by Step Setup worktable for preparation of 12-in-1 rack.
8. Place the Multi-Sample DNA Extraction Cartridges (DS0399) on 12-in-1 rack as shown in the below figure.

Fig. 6

9. Place the columns (DBCA021) on the cartridge as shown below:

Fig. 7

10. Place 1ml tip (DS0337) set onto the 12-in-1 rack.

Fig. 8
11. Place Collection tube, Polypropylene (2.0 ml) (PW1139) on 12-in-1 rack and close the metal lid.

![Fig. 9](image)

12. Place the 12-in-1 rack into the instrument as shown in the below fig. and secure the rack in position by two lock plates aside the worktable.

![Fig. 10](image)


**Sample Pre-treatment procedure**

I. **Preparation of Forensic Sample**
   Prepare the following forensic samples according to the steps given below:
   a. Cigarette butts
   b. Envelopes, stamps, chewing gum or epithelial cells on fabric
   c. Hair roots
   d. Nail clippings or soft tissue
   e. Material stained with blood, saliva or semen

   a. **To lyse cigarette butts**
      Cut a 1cm² piece of outer paper from the end of the cigarette or filter. Cut it further into 6 smaller pieces and transfer the pieces into a 2.0ml capped microcentrifuge tube.

   b. **To lyse envelopes, stamps, chewing gum, epithelial cells on fabric**
Cut a 0.5 - 2.5 cm² sample from the envelope or stamp. Cut 0.5 - 2.5 cm² chewing gum or fabric into small pieces. Cut it further into smaller pieces and transfer the pieces into a 2.0ml capped microcentrifuge tube.

c. **To lyse hair roots**
   Cut a 0.5 - 1.0 cm piece starting from the hair bulb. Transfer the piece into a 2.0ml capped microcentrifuge tube.

d. **To lyse nail clippings or soft tissue**
   Clip the nails into small pieces and chop soft tissues (approximately up to 25mg) to fine pieces. Transfer the pieces into a 2.0ml capped microcentrifuge tube.

e. **To lyse material stained with blood, saliva or semen**
   Take 0.5 cm² area of the stained material and cut into smaller pieces. Transfer the pieces into a microcentrifuge tube.

**NOTE: Continue with step II of DNA Extraction Protocol (1a to 1e)**

**DNA Extraction Protocol (1a to 1e):**

II. **Lysis of Forensic Sample**

Add 300 µl Lysis Solution AL (DS0015), 20 µl Proteinase K (20 mg/ml) ([Refer to General preparation Instructions](#)) and 20 µl of 1M DTT ([Refer to General preparation Instructions](#)) to the material in the collection tube. Mix thoroughly by pulse-vortexing for 10-15 seconds and incubate at 55°C for 15 min.

III. Centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 1 minute at room temperature. Take out the supernatant in new capped collection tube and proceed with step 14.

14. Place the Collection tube containing sample onto the 12-in-1 rack.

![Fig. 11](image-url)
15. Close the front door. Click **Start Run**.

![Fig. 12](image12.png)

16. Following screen will appear while the run is going on.

![Fig. 13](image13.png)

17. After the run is completed, the screen will look like the below figure.

![Fig. 14](image14.png)
18. Lift the instrument lid and take out the 12-in-1 rack. Remove the elution tubes and discard all other plastic wares.

19. The eluted DNA can be further used for PCR amplification.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs.) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

**Warning and Precautions**
Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

**Limitations**
1. The yield of DNA depends upon the type and the quantity of starting material used.

**Performance and Evaluation**
Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

**Quality Control**

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>DNA Yield</th>
<th>DNA Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal swab</td>
<td>4-12 μg</td>
<td>1.6-1.9</td>
</tr>
</tbody>
</table>

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*Please refer disclaimer Overleaf.*
Troubleshooting Guide

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Poor / Lower yield of genomic DNA</td>
<td>Sample is too old</td>
<td>Yield of genomic DNA varies from sample to sample. We recommend using fresh sample.</td>
</tr>
<tr>
<td>2.</td>
<td>Purity of the DNA is lower than expected; A&lt;sub&gt;260&lt;/sub&gt;/A&lt;sub&gt;280&lt;/sub&gt; ratio is too high.</td>
<td>RNA contamination</td>
<td>RNase A treatment should be included in future isolations or the final product can be treated with RNase A and repurified.</td>
</tr>
</tbody>
</table>

Safety Information

Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.

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Disclaimer:

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