Forensic Buffer

Product Name	Product Code	Kit Packing
Forensic Buffer	ML128-500ML	500 ml

Intended Use

Recommended for extraction of DNA from Forensic samples.

Introduction:

Forensic Buffer is used to extract DNA from various forensic samples like stained blood, saliva or semen, cigarette butts, buccal swabs, envelopes, hair, nail clippings, stamps etc.

Description:

In forensic study DNA has to be isolated from wide range of biological samples which include blood, soft tissue, semen, urine, saliva, teeth etc. Forensic samples are routinely found as stains on various substrates like cotton, wood, envelopes, cigarette butts etc. Forensic Buffer is used to extract DNA from blood and other biological samples. This buffer lyses the red blood cells (erythrocytes) and helps to remove the DNA from other cellular components. Forensic Buffer contains EDTA which inactivates nucleases by chelating metal cations.

Application:

Forensic Buffer is extensively used to extract DNA from blood and other biological samples in forensic studies.

Properties:

Appearance	:	Colorless solution
Clarity	:	Clear and free of particles
DNase/ RNase	:	None detected
Sterility	:	No Bacterial or Fungal growth observed after 14 days of incubation as per USP Specifications
Suitability test	:	This solution has been tested and is suitable for use in for use in forensic applications.

Storage:

Forensic Buffer has to be stored at 15-25°C. Under recommended condition, the product is stable for 48 months.

Types of Specimen

Forensic samples like stained blood, saliva or semen, cigarette butts, buccal swabs, envelopes, hair, nail clippings, stamps etc.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Materials Required but not provided

1. Lysis Solution (C1) (Product Code: DS0010)
2. Prewash Solution Concentrate (PW) (Product Code: DS0011) – Dilute the Prewash Solution concentrate in a ratio of 2:3 with 100 % Ethanol, prior to use.
3. Wash Solution Concentrate (WS) (Product Code: DS0012) - Dilute the Wash Solution concentrate in a ratio of 1:3 with 100 % Ethanol, prior to use.
4. Elution Buffer (ET) [10mM Tris-Cl, pH 8.5 (Product Code: DS0040)
5. Proteinase K Solution (20mg/ml) (Product Code: DS0013)
6. 1M DL-Dithiothreitol (Product Code: ML205)
7. Ethanol (96 – 100%)
8. Molecular Biology Grade Water (Product Code: ML024)
9. Table Top Centrifuge
10. Heating block at 55°C
11. Heating block at 70°C
12. HiElute Miniprep Spin Column (Product Code: DBCA03)
13. Collection Tube, 2.0ml (Product Code: PW147)
14. HiPurA® Forensic Multisample DNA Purification Kit (Product Code: MB580)

General Preparation Instructions

1. Set the heating block or thermomixer at 55°C.
2. 1M DTT has to be stored at -20°C in dark.
3. Examine Stain Extraction Buffer for precipitation. If precipitate observed, warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.
4. Ensure that clean & dry tubes and tips are used for the procedure.

Procedure

1. Preparation of blood-stained cloth for DNA Extraction

a) Take 0.5cm^2 area of the stained material and cut into smaller pieces. Transfer the pieces into a microcentrifuge tube. Add 300 µl of Forensic Buffer, 20µl, 1M DTT and 20µl Proteinase K Solution (20mg/ml). Incubate at 55°C for 1- 1½ hours with intermittent mixing or shaking at 900rpm in thermomixer.

b) Spin the tube at 12,000rpm for 2 minutes.

c) Carefully discard the cloth and transfer the supernatant to a fresh 2.0 ml Collection Tube. Proceed with Lysis step.

2. Lysis reaction

Add 300 µl of the Lysis Solution (C1) (DS0010) to the sample, vortex thoroughly for a few seconds to obtain a homogenous mixture. Incubate at 70^{o}C for 10 minutes.

NOTE: If cell clumps are visible, the sample can be mixed gently by pipetting to obtain a homogenous mixture.

3. **Prepare for Binding**

   Add 300 µl of ethanol (96-100%) to the lysate obtained from the above step for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.
   NOTE: A homogenous solution is essential.

4. **Load lysate in HiElute Miniprep Spin Column (Capped)** [**DBCA03]**

   Transfer the lysate obtained from step 3 into the spin column provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.
   NOTE: Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

5. **Prewash**

   Add 500 µl of Prewash Solution diluted to the column and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

6. **Wash**

   Add 500 µl of Wash Solution diluted to the column and centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed if residual ethanol is observed. Discard the collection tube containing the flow through liquid and place the column in a new uncapped 2.0 ml collection tube.
   NOTE: The column must be free of ethanol before eluting the DNA. The tube can be emptied and re- used for this additional centrifugation step.

7. **DNA Elution**

   Pipette 20 - 50 µl of the Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

   NOTE: DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time (DNA yield would be low). Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25^{0}C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

8. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

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.

Specimen Handling and Collection

The collection and preservation of blood stain evidence is important because, properly collected and preserved blood evidence can establish a strong link between an individual and a criminal act. There are two different types of blood that can be collected at a crime scene: liquid and dried blood. Liquid blood evidence is generally collected from blood pools but can be collected off of clothing as well, using a gauze pad or a sterile cotton cloth. After use, contaminated material must be sterilized by autoclaving before discarding.

Limitations

The yield of DNA depends upon the type and the volume of starting material used.

Performance and Evaluation

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

Safety Information

The Forensic Buffer is for laboratory use only, not for drug, household or other uses. This reagent contains detergent which may cause irritation to skin and eyes. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance

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

Symbol

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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