# Stain Extraction Buffer

| Product Name | Product Code | Kit packing |
|---|---|---|
| Stain Extraction Buffer | ML127-500ML | 500 ml |
| | ML127-2X500ML | 2X500 ml |

**Intended Use**

Recommended for extraction of DNA from Forensic samples.

**Introduction:**

Stain Extraction Buffer is used to extract DNA from stained blood, saliva or semen, cigarette butts, buccal swabs, envelopes, hair, nail clippings, stamps etc.

**Application:**

Stain Extraction Buffer is extensively used to extract DNA from blood stained cloth / filter paper samples. This solution has to be supplemented with DTT before use.

**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:**

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

**Types of Specimen**

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

**Materials needed but not provided**

- Lysis Solution (C1) (Product Code: DS0010)
- Prewash Solution Concentrate (PW) (Product Code: DS0011) – Dilute the Prewash Solution concentrate in a ratio of 2:3 with 100 % Ethanol, prior to use.
- Wash Solution Concentrate (WS) (Product Code: DS0012) - Dilute the Wash Solution concentrate in a ratio of 1:3 with 100 % Ethanol, prior to use.



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Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 0 0-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

- Elution Buffer (ET) [ 10mM Tris-Cl, pH 8.5 (Product Code: DS0040)
- Proteinase K Solution (20mg/ml) (Product Code: DS0013)
- 1M DL-Dithiothreitol (Product Code: ML205)
- Ethanol (96 – 100%)
- Molecular Biology Grade Water (Product Code: ML024)
- Table Top Centrifuge
- Heating block at 55°C
- Heating block at 70°C
- HiElute Miniprep Spin Column (Product Code: DBCA03)
- Collection Tube, 2.0ml (Product Code: PW147)
- 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 cloth stained with blood, saliva or semen for DNA Extraction**
   Take 0.5cm$^2$ area of the stained material and cut into smaller pieces. Transfer the pieces into a microcentrifuge tube. (Continue with Step 2)

2. **Lysis**
   Add 300 µl Stain Extraction Buffer, 20 µl Proteinase K (20 mg/ml) and 20 µl of 1M DTT to the material in the collection tube. Mix thoroughly by pulse-vortexing for 10-15 seconds.

3. Incubate at 55°C for atleast 1- 1½ hours with shaking at 900 rpm in a thermomixer.
   **NOTE:** If using a heating block or water bath, vortex the tube after every 10 minutes for 10-15 seconds to improve lysis of the sample material.

4. Add 300 µl of Lysis Solution C1 (DS0010) and mix thoroughly by pulse-vortexing for 10-15 seconds.
   **NOTE:** To ensure efficient lysis, mix the sample and Lysis Solution C1 thoroughly to form a homogenous solution. A white precipitate may form when Lysis Solution C1 is added to Lysis Solution AL. This precipitate will dissolve during incubation in Step 5.

5. Incubate at 70°C for 10 minutes with shaking at 900 rpm in a thermomixer.
   **NOTE:** If using a heating block or water bath, vortex the tube after every 3 minutes for 10-15 seconds to improve lysis of the sample material.

6. Centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 1 minute at room temperature.

7. **Prepare for Binding**
   Transfer the supernatant carefully, obtained from step 6, into a new 2.0 ml collection tube (not provided) and add 300 µl of ethanol (96- 100%) for preparation of the lysate for binding. Mix thoroughly by vortexing for 5-10 seconds.

8. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Add 650 µl of the mixture from including any precipitate, which may have formed, to the HiElute Miniprep Spin Column (Capped). Centrifuge for 1 minute at 6000 x g (≈8000 rpm) at room temperature. Discard the flow-through.
   **NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

9. Repeat step 8 with the remaining sample. Discard the flow-through liquid.

10. **Prewash**
    **(Prepare the Prewash Solution (PW) (DS0011) as indicated in General Preparation Instructions)**
    Place the column in a same 2.0 ml collection tube and add 700 µl of diluted Prewash Solution (PW) to the column. Centrifuge at 6,500 x g (≈10,000 rpm) for 1 minute at room temperature (15-25°C). Discard the flow-through liquid and re-use the same collection tube with the column.

11. **Wash**
    **(Prepare the Wash Solution (WS) (DS0012) as indicated in General Preparation Instructions)**
    Add another 700 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin Column (Capped) and centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 1 minute at room temperature. Discard the flow-through liquid and reuse the same collection tube.

12. Centrifuge the column for 2 minutes at 20,000 x g (≈14,000 rpm) to dry the column membrane to remove the traces of residual ethanol, if observed. Place the column in a new uncapped 2.0 ml collection tube.

13. **DNA Elution**
    Pipette 20 - 50 µl of the Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the center of the HiElute Miniprep Spin Column (Capped) membrane without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA.

    **NOTE:** To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer, then centrifuge. Elution with volumes less than

50 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

14. 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 Stain Extraction Buffer is for laboratory use only, not for drug, household or other uses. 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**

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|---|---|---|---|
|  | 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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