Elution Buffer (ET) (10mM Tris-Cl, pH8.5)

Product Name	Product Code	Kit Packing
Elution Buffer (ET)	DS0040-250ML	250 ml
(10mM Tris-Cl, pH8.5)	DS0040-500ML	500 ml

Intended Use

Recommended for elution of DNA from human blood, plasma, tissue and serum samples.

Introduction: Elution Buffer (ET) (10mM Tris-Cl, pH8.5) is a buffer solution used for the purpose of eluting cells/ tissues for use in molecular biology experiments.

Application: Elution Buffer (ET) (10mM Tris-Cl, pH8.5) is used for eluting blood cells, animal cells, tissues and bacterial cells from membrane.

Properties:

Appearance: Colorless solution
Clarity: Clear and free of particles
DNase & RNase: None detected
Suitability Test: This reagent has been tested and is suitable for elution of DNA from blood, animal cells tissues and bacteria etc.

Storage conditions: Elution Buffer (ET) (10mM Tris-Cl, pH8.5) has to be stored at 15 - 25°C. The shelf- life of this solution in 12 months.

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 tissues in a sterile container. Store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the blood sample/ tissues are 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, Plasma, Serum, cells and tissues

Procedure

1. **Collect Blood**

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

For Frozen blood: To 200 µl of frozen blood pellet (kept on ice), add 200 µl of Lysis Solution (C1) (DS0010). Thaw the pellet with continuous pipetting and proceed with step 2 for Proteinase K and RNase A treatment (optional). Incubate at 55°C for 10 minutes and then proceed to step 4 of the protocol.

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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 : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

NOTE: When extracting DNA from frozen blood, it is very important that the blood should be kept on ice and directly mixed with Lysis Solution (C1). Do not allow the frozen blood pellet to thaw except when it is directly mixed with Lysis Solution (C1). This prevents release of apoptotic enzymes that can decrease the DNA yield drastically.

NOTE: If the sample is less than 200 µl, add the Resuspension solution (ML116) to bring the volume upto 200 µl.

Procedure

Add 20 µl of the Proteinase K solution (20 mg/ml) (DS0013) into 2.0 ml capped collection tube containing 200 µl of the whole blood. Vortex for 10-15 seconds to ensure thorough mixing.

Optional RNase A treatment
If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003). Vortex for 10-15 seconds and incubate for 2 minutes at room temperature (15-25^{o}C); continue with step 3.

1. **Lysis reaction**
   Add 200 µl of the Lysis Solution (C1) (DS0010) to the sample, vortex thoroughly for a few seconds to obtain a homogenous mixture. Incubate at 55^{o}C for 10 minutes.

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

2. **Prepare for Binding**
   Add 200 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.

3. **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.

4. **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.

5. **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.

6. **DNA Elution**
   Pipette 100 µl of the Elution Buffer (ET) (DS0040) 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.

7. 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.

Limitations

1. 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.

Quality Control

Type of Sample	DNA Yield	DNA Purity
200 µl whole blood	4-12 µg	1.6-1.9

Safety Information

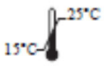
The Elution Buffer (ET) (10mM Tris-Cl, pH8.5) contains irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

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.



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com

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HiMedia Laboratories Pvt. Ltd. Reg.office : 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: 00-91-22-6147 1919, 6903 4800 Email: techhelp@himedialabs.com Website: www.himedialabs.com