HiGenoMB® HIMEDIA
Unzipping Genes

HIMEDIA®
Product Information

Lysis Solution (AL)

Product Name	Product Code	Kit Packing
Lysis Solution (AL)	DS0015-200ML	200 ml

Intended Use:

Recommended for isolation of DNA from cells and tissue samples of human and animal origin.

Introduction:

Lysis Solution (AL) is a buffer solution used for the purpose of lysing cells and tissues for use in molecular biology experiments. This solution contains a detergent to break up the membrane structures.

Application:

Lysis Solution (AL) is used for lysing tissues and cells to extract DNA.

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 extraction of DNA from human/ animal cells and tissues, bacteria etc.

Storage conditions: Lysis Solution (AL) has to be stored at 15 - 25°C. The shelf- life of this solution in 12 months.

Specimen Handling and Collection

Collect human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Ensure that the tissue is at room temperature before beginning the protocol.

Types of Specimen

Clinical samples: human tissue
Animal tissue: liver, lungs, muscle

A. Mammalian Tissue Preparation

1. **Prepare tissue**
   Weigh a piece of fresh or frozen tissue and mince quickly. If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation. Cut the tissue into small pieces as it enables more efficient lysis. Up to 25 mg of tissue (or 10 mg of spleen, due to the high number of cells per given mass) may be used per preparation. Transfer to a given capped 2.0 ml collection tube and continue to step 2 of Mammalian Tissue Preparation.

   NOTE: Tissue can be harvested, by aliquoting in 2.0 ml collection tubes and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing DNA.
2. **Digest tissue**

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Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution (20 mg/ml) to the tissue. Mix by vortexing. Incubate the sample at 55°C until the tissue is completely digested with no particles remaining. Mix by vortexing occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. Vortex briefly after digestion is completed.

Optional RNase A treatment
If RNA-free genomic DNA is required, add 20 µl of RNase A solution (DS0003), mix and incubate for 2 minutes at room temperature (15-25°C), then continue with step 3 of Mammalian Tissue Preparation.

3. **Lyse cells**
   Add 200µl of Lysis Solution (C1) (DS0010) to the sample. Mix by vortexing thoroughly for 15 seconds. A homogeneous mixture is essential for efficient cell lysis. Incubate at 70°C for 10 minutes.

4. **Binding**
   Add 200 µl of ethanol (96-100%) to the lysate and mix thoroughly by vortexing for 5-10 seconds.

   NOTE: A homogeneous solution is essential. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the column. This precipitate does not interfere with the DNA isolation procedure or with any subsequent application. Do not use alcohols other than ethanol because this may result in reduced yields.

5. **Load lysate in HiElute Miniprep Spin Column (Capped)[DBCA03]**
   Transfer the lysate obtained from step F onto the 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. It is essential to apply all of the precipitate to the HiElute Miniprep Spin Column. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through. Centrifugation at ≥14,000 rpm will not affect the yield or purity of the DNA.

6. **Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution 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.

7. Add another 500 µl of diluted Wash Solution to the column and centrifuge at 12,000 – 16,000 g (≈13,000-16,000 rpm) for 3 minutes to dry the column. Centrifuge the 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.

8. **DNA Elution**

Pipette 200 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.

Optional: A second elution can be collected by repeating step 9.

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

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer, then centrifuge. Elution with volumes less than 200 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.

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 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
30 mg of tissue	30-45 µg	1.6-1.9

Safety Information

The Lysis Solution (AL) contains chaotropic salts, which are 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.

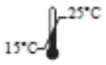
Please refer disclaimer Overleaf.

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

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