MB566 RDP Trio™ Reagent

Product Code

MB566- 100 ml (100 ml RDP Trio™ Reagent)
MB566- 5 X 100 ml (5X100 ml RDP Trio™ Reagent)

Intended Use

Recommended for isolation of DNA, RNA and Protein from tissue, plant, and cells.

Storage Conditions

Store at 15-25°C

Materials needed but not provided

For RNA Isolation

- Chloroform (Product Code: MB109)
- Isopropyl alcohol (Product Code: MB063)
- 75% Ethanol (in DEPC-treated water)
- 0.5% SDS (Product Code: ML007 – 20% SDS Stock solution).
- Molecular Biology Grade Water (RNase free) (Product Code: ML024)

For DNA Isolation

- Ethanol (96 - 100%)
- 0.1M trisodium citrate, 10% Ethanol solution
- Elution Buffer (ET) (10mM Tris-Cl, pH 8.5 (Product Code: DS0040)

For Protein Isolation

- Isopropyl alcohol (Product Code: MB063)
- 0.3M Guanidine Hydrochloride, 95% Ethanol
- Ethanol (96 - 100%)
- 1% SDS (Product Code: ML007 – 20% SDS Stock solution)

Introduction

RDP Trio™ Reagent is a quick and convenient reagent to use in the isolation of RNA, DNA and Proteins from human (tissue), animal and plant samples. The protocol is rapid and permits isolation from large number of samples of small or large volumes. The RNA obtained can be further used for downstream applications such as Northern blot, mRNA isolation, *in vitro* translation, RNase and S1nuclease protection assay, RT-PCR and cloning. The procedure is very effective for isolating RNA molecules of all types from 0.1 to 15 kb in length. DNA isolated by using RDP Trio™ Reagent is suitable for PCR, restriction enzyme digestion and Southern blotting, whereas, Protein isolated using RDP Trio™ Reagent can be probed for specific proteins by Western Blotting.

Principle

HiMedia's RDP Trio™ Reagent is designed for rapid purification of RNA, DNA and Protein from different samples. This product which is a mixture of guanidine thiocyanate and phenol in a mono-phase solution effectively dissolves RNA. After adding chloroform and centrifuging, the mixture separates into 3 phases: an aqueous phase containing the RNA, the interphase containing DNA and an organic phase containing protein. 1 ml of RDP

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Trio™ Reagent is sufficient to isolate RNA, DNA and Protein from 50-100 mg of tissue, 5-10X10^6 cells or 10 cm^2 of culture dish surface, for cells grown in monolayer. This advanced procedure is an improvement to the single-step RNA isolation using phenol and guanidine isothiocyanate developed by Chomczynski and Sacchi. This is one of the most effective methods for isolating total RNA and can be completed in only 1 hour starting with fresh tissue and cells.

Storage

Store RDP Trio™ Reagent between 15-25°C. Under recommended condition this reagent is stable for 1 year.

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even trace amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.

3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH and 1mM EDTA followed by RNase-Free Water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours before use. Alternatively glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC treated water, allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-Free Water, and then rinsed with ethanol and allowed to dry.

6. Solutions (water and other solutions) should be treated with 0.1% DEPC.

Specimen Handling and Collection

<u>For Plant</u>

Collect plant tissue in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

<u>For Cells and tissues</u>

Collect cells and tissues 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: Cells, Tissues and Plant

Protocol

1. **Sample Preparation**

a. **Tissue:**
Homogenize tissue samples in RDP Trio™ Reagent (1 ml for 50-100 mg of tissue) in a Homogenizer with serrated pestle S.P-2 ml (Product Code-GW117) or other appropriate homogenizer.

NOTE: The amount of tissue should not exceed 10% of the volume of RDP Trio™ Reagent

b. **Monolayer Cells:**
Add 1 ml of RDP Trio™ Reagent per 10 cm^2 of glass culture plate surface area to lyse cells. After addition of the reagent, the cell lysate should be mixed thoroughly using a micropipette to form a homogenous lysate.

NOTE: RDP Trio™ Reagent is not compatible with plastic culture plates.

c. **Suspension cells:**
Pellet up to $1x10^7$ cells by centrifuging at 4°C for 5 minutes at 300 x g (≈1500 rpm) in a collection tube (not supplied). Discard supernatant and then lyse pellet in RDP Trio™ Reagent by repeated pipetting. 1 ml of reagent is sufficient to lyse upto $10X10^6$ animal, plant or yeast cells or 10^7 bacterial cells.

NOTE:

A. Some yeast and bacterial cells may require a homogenizer
B. After the cells have been homogenized or lysed in RDP Trio™ Reagent, samples can be stored at –70°C for up to 1 month.
C. Samples containing high amount of fat, protein, polysaccharides or extracellular material (muscle, fat tissue and tuberous parts of plants) may require centrifugation at 12,000 x g (≈13,000 rpm) for 10 minutes at 4°C after homogenization. The supernatant contains RNA and Protein. If the sample had a high fat content there will be a layer of fatty material on the surface of the aqueous phase that should be removed. Transfer the clear supernatant to a fresh tube and proceed with step 2.

2. **Phase separation**
Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes. Add 200µl of Chloroform per ml of RDP Trio™ Reagent used. Cover the sample tightly, shake vigorously for 15 seconds, and allow to stand for 10 minutes at room temperature (15-25°C). Centrifuge the resulting mixture at 12,000 x g (≈13,000 rpm) for 15 minutes at 4°C. Following centrifugation, mixture separates into lower deep red organic phase (containing protein), an interphase (containing DNA) and a colorless upper aqueous phase containing RNA. [Continue with respective PROCEDURE FOR ISOLATING RNA/DNA/PROTEIN]

NOTE: The chloroform used for phase separation should not contain Isoamyl alcohol and any other additives.

PROCEDURE FOR ISOLATING RNA

RNA Precipitation

1. Transfer the aqueous phase containing RNA to a fresh tube and add 500µl of Isopropyl alcohol per ml of RDP Trio™ Reagent used. Mix the sample thoroughly by repeated pipetting. Allow the sample to stand for 5-10 minutes at room temperature (15-25°C). Centrifuge at 12,000 x g (≈13,000 rpm) for 10 minutes at 4°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

   NOTE: Store interphase and organic phase at 2-8°C for isolation of DNA and Proteins.

2. **RNA Wash**

   Remove the supernatant without disturbing the pellet. Wash the RNA pellet by adding 1 ml of 75% ethanol per ml of RDP Trio™ Reagent used. Vortex the sample and then centrifuge at 7,500 x g (≈10,500 rpm) for 5 minutes at 4°C.

   NOTE:

a. If the RNA pellets float, perform aditional wash in 75% ethanol at 12,000 x g (≈13,000 rpm).
b. Samples can be stored in ethanol for atleast 1 week at 4°C and upto 1 year at -20°C at this step.

3. **Redissolving the RNA**

   Discard the supernatant without disturbing the pellet. Briefly dry the RNA pellet for 5-10 minutes by air-drying or under a vaccum.

   NOTE: Do not let the RNA pellet dry completely, as this will greatly decrease its solubility. Do not dry the RNA pellet by centrifugation under vacuum.

   Add an appropriate volume (50µl) of RNase-Free Water to the RNA pellet. To facilitate dissolution, mix by repeated pipeting with a micropipette. Incubate at 55-60°C for 10-15 minutes.

   Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

   NOTE: In rare occasions, when traces of DNA are observed, we recommend digesting the RNA with RNase-free DNase, if desired.

PROCEDURE FOR ISOLATING DNA

DNA Precipitation

1. Discard the remaining aqueous phase overlaying the interphase. Transfer the interphase containing DNA to a fresh tube and add 300 µl of 100% Ethanol per ml of RDP Trio™ Reagent used. Mix by inversion and allow the sample to stand for 2-3 minutes at room temperature (15-25°C). Centrifuge at 12,000 x g (≈13,000 rpm) for 5 minutes at 4°C.

2. **DNA Wash**

   Remove the supernatant without disturbing the pellet and store at 2-8°C for protein isolation. Wash the DNA pellet by adding 1 ml of 0.1M trisodium citrate, 10% Ethanol solution. Allow the DNA pellet to stand for 30 minutes with occasional mixing at room temperature (15-25°C). Centrifuge at 7,500 x g (≈10,500 rpm) for 5 minutes at 4°C. Discard supernatant and repeat this wash step once.

Discard supernatant and dissolve the DNA pellet in 1.5 ml of 75% Ethanol. Allow to stand for 10-20 minutes at room temperature (15-25°C). Centrifuge at 7,500 x g (≈10,500 rpm) for 5 minutes at 4°C.

NOTE: Samples can be stored in Ethanol for several months at 2-8°C

3. **Redissolving the DNA**
   Discard the supernatant without disturbing the pellet. Briefly dry the DNA pellet for 5-10 minutes by air-drying or under a vaccum. Add an appropriate volume (100µl) of Elution Buffer (ET) (10mM Tris-Cl, pH 8.5) (DS0040) to the DNA pellet. Centrifuge at 7,500 x g (≈10,500 rpm) for 10 minutes at 4°C to remove any insoluble material. Transfer the supernatant to a new tube.

   Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short- term storage (24-48 hours) 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 (ET) will help to stabilize the DNA at these temperatures.

PROCEDURE FOR ISOLATING PROTEINS

Protein Precipitation

1. Precipitate proteins from the supernatant isolated in step 2 of DNA Isolation procedure. Add 1.5 ml of Isopropanol per ml of RDP Trio™ Reagent used. Allow the sample to stand for 10 minutes at room temperature (15-25°C). Centrifuge at 12,000 x g (≈13,000 rpm) for 10 minutes at 4°C.

2. **Protein Wash**
   Discard the supernatant without disturbing the pellet. Wash the pellet by adding 2 ml of 0.3M guanidine hydrochloride, 95% ethanol solution. Allow the pellet to stand for 20 minutes at room temperature (15-25°C). Centrifuge at 7,500 x g (≈10,500 rpm) for 5 minutes at 4°C. Discard supernatant and repeat this wash step twice.
   Discard supernatant and dissolve the protein pellet by vortexing the pellet in 2 ml of 96-100% Ethanol. Allow the sample to stand for 20 minutes at room temperature (15-25°C). Centrifuge at 7,500 x g (≈10,500 rpm) for 5 minutes at 4°C.

4. **Redissolving the Protein**
   Discard the supernatant without disturbing the pellet. Briefly dry the Protein pellet for 5-10 minutes by air-drying or under a vaccum. Add an appropriate volume of 1%SDS to the protein pellet. Centrifuge at 7,500 x g (≈10,500 rpm) for 10 minutes at 4°C to remove any insoluble material. Transfer the supernatant to a new tube.

   Storage of the eluate with purified Protein: The extracted protein solution can be used immediately for Western blotting or stored at-20^{O}C.

References

1. Chomczynski P., BioTechniques 15, 532-537(1993).
2. Chomczynski P. and Sacchi,N., Anal .Biochem.,162,156-159(1987)

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 laboratory practices while handling 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

Each lot of HiMedia's RDP Trio™ Reagent is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Purity	RNA Purity
CHO cells	1.6-1.9	2.0
Plant tissue	1.6-1.9	2.0

Trouble Shooting Guide

For RNA Isolation:

Sr. No.	Problem	Sample	Cause	Solution
1.	Low yield of RNA	Any sample	Incomplete homogenization or lysis of samples	No particulate matter should remain in the tube. Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes.
			RNA pellet was not dissolved completely	To facilitate dissolution, mix by repeated pipetting with a micropipette at 55-60°C for 10-15 minutes.
2.	Low A_{260}/A_{280}	Any sample	The final RNA pellet may not have been dissolved completely	Dissolve the RNA pellet completely in RNase-Free Water by pipetting.
			Samples may not have been allowed to stand for 5 minutes after homogenization	Allow the samples to stand at room temperature for 5 minutes before proceeding with chloroform step.
			Sample used for isolation of RNA may be too small	Use correct amount of sample as mentioned in step 1 of the RNA isolation protocol.
			Contamination of aqueous phase with phenol phase	Carefully pipette out aqueous phase only.
3.	RNA degradation	Any sample	Tubes or solutions used	Use RNase free tubes and solutions.

			for RNA isolation may not have been RNase free	
			Formaldehyde used for preparation of agarose gel may have a pH <3.5	Verify that the pH of Formaldehyde is >3.5
4.	DNA contamination	Any sample	Volume of reagent used may be too small	Volume of reagent should be used accordingly as mentioned in the product insert.

For DNA Isolation:

Sr. No.	Problem	Sample	Cause	Solution
1.	Low yield of DNA	Any sample	Incomplete homogenization or lysis of samples	No particulate matter should remain in the tube. Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes.
			DNA pellet was not dissolved completely	To facilitate dissolution, mix by repeated pipetting with a micropipette at 55-60°C for 10-15 minutes.
2.	Low A_{260}/A_{280}	Any sample	Phenol is not completely removed from DNA preparation	Wash the DNA pellet one more time by adding 1 ml of 0.1M trisodium citrate, 10% ethanol solution.
3.	DNA degradation	Any sample	Tissues are not immediately processed or frozen	Proceed immediately with the protocol or freeze the tissues.
4.	RNA contamination	Any sample	DNA pellet may not be washed sufficiently	Wash DNA pellet sufficiently with 0.1M trisodium citrate, 10% ethanol.
			Contamination of aqueous phase with organic phase	Carefully pipette out organic phase only.

For Protein Isolation:

Sr. No.	Problem	Sample	Cause	Solution
1.	Low yield of Protein	Any sample	Incomplete homogenization or lysis of samples	No particulate matter should remain in the tube. Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes.
			Protein pellet was not dissolved completely	To facilitate dissolution, mix by repeated pipetting with a micropipette at 55-60°C for 10-15 minutes.
2.	Protein degradation	Any sample	Tissues are not immediately processed or frozen	Proceed immediately with the protocol or freeze the tissues.
3.	Band deformation in PAGE	Any sample	Protein pellet may not be washed sufficiently	Wash protein pellet sufficiently with 0.3M guanidine hydrochloride, 95% ethanol solution

Safety Information

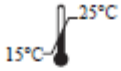
HiMedia's RDP Trio™ Reagent is for laboratory use only, not for drug, household or other uses. This product which is a mixture of guanidine thiocyanate and phenol in a mono-phase solution effectively dissolves RNA. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. 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

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