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TriFast II

ready-to-use
solution for the simultaneous isolation of RNA,DNA and proteins

For research use only

Cat.	Size
EMR517100	100 ml
EMR517200	2 X 100 ml

Storage and Stability: 6 months at 2-8°C. Protect from light.

Introduction

TriFast II is a complete ready to use mono-phasic reagent for the simultaneous isolation of RNA, DNA and proteins from solid and liquid samples of human, animal, plant, yeast, bacterial and viral origin. The isolation method for RNA, DNA and proteins is built on the well-known single step liquid phase separation (RNAClean™), with additional modifications to improve the purity of the RNA, DNA and proteins. This method has been shown to yield non-degraded nucleic acids (RNA and DNA) from small and large samples and allow the isolation of large and small RNA-species (0.1 - 15 kb) with high purity. Also it makes possible to process a large number of samples simultaneously.

TriFast II includes phenol and guanidinium thiocyanate in a monophasic solution. After addition of chloroform, the homogenate separates into three phases upon centrifugation. RNA will be selectively retained in the aqueous phase, while DNA and proteins stay respectively in the organic phase and interphase and are processed separately, resulting in RNA, DNA and protein of excellent quality in high yield. Simultaneous purification of DNA is an effective way to normalize RNA yields from sample to sample.

IMPORTANT NOTES

- Hands and dust in the laboratory can be the major source for RNase contaminations. Use gloves and keep the tubes closed during RNA preparation.
- To facilitate isolation of RNA from small samples (<10^6 cells or <10 mg tissue) perform homogenization (or lysis) in the presence of 70 µg of glycogen carrier per 1 ml TriFast II used.
- After homogenization i.e. before the addition of chloroform samples can be stored at -70 °C for a few months. The RNA precipitate from step 1.4 can be stored in 75% ethanol for 1-3 weeks at 4°C or for at least one year at -20°C.
- For samples with high content of proteins, polysaccharides, fat or other materials an extra purification step should be included. After homogenization do not proceed to phase separation with chloroform but rather remove insoluble material by centrifugation at 12.000 g for 10 minutes at 4°C. The supernatant contains RNA while the pellet consists of polysaccharides, extracellular membranes and high molecular weight DNA. Samples from fat tissues will result in a floating fat layer that should be removed. Transfer the clear RNA supernatant to a fresh tube and proceed with the phase separation as described. High molecular weight DNA can be isolated from the pellet by following steps 2.2 and 2.3 of the DNA isolation protocol.

Protocol

1.) Method for RNA isolation

TriFast II isolates the whole range of RNAs including mRNA and rRNA. The RNA isolation procedure can be completed in about 1 hour. The yield of RNA is comparable or higher than with any other method. The total RNA isolated by TriFast II is undegraded and free of protein and DNA. The RNA can be used for dot blot hybridization, poly A+ selection, in vitro translation, molecular cloning, RNase protection assays, Northern analysis and for PCR reactions. Since genomic DNA can be extracted from the same material Northern data or other can be normalized to DNA instead of more variable values like RNA or even tissue weight.

Reagents required, but not supplied

- Chloroform,
- Isopropanol
- Ethanol
- Disposable polypropylene tubes of high quality should be used for work with TriFast II (centrifugation at 12.000 g in the presence of phenol!)

1.1) Homogenization

a. Tissue samples: Homogenize tissue samples in 1ml TriFast II per 50-100 mg tissue. For efficient lysis use a glass-Teflon or power homogenizer. The sample volume should not exceed 10% of the volume of TriFast II used for the homogenization.
b. Cells grown in monolayer: After aspiration of the medium, lyse cells directly in a culture dish by addition of 1 ml TriFast II to a 3.5 cm diameter dish and passing the cell lysate several times through a pipette. The amount of TriFast II needed is based on the area of the culture dish (1 ml per 10 cm^2) and not on the number of cells. An insufficient amount of TriFast II may result in contamination of the isolated RNA with DNA.
c. Cells grown in suspension: Pellet cells by centrifugation. Lyse cells in TriFast II by pipetting. Add 1 ml reagent per 1-5 x 10^6 cells or up to 1 x 10^7 of bacteria cells.
d. Blood samples, serum or other biological fluids: Add 750 µl of TriFast II per 250 µl of sample volume.

> **Note**: Biological fluids with high levels of protein or other contaminating substances (e.g. whole blood) may be diluted 1:1 with RNase-free, molecular biology grade water.

Homogenize the sample by pipetting the suspension up and down several times. A short incubation period (5 minutes) at room temperature improves the separation of RNA / protein complexes

1.2) Phase separation

Add 0,2 ml of chloroform to the lysate or, alternatively, 0,1 ml of 1-Bromo-3-chloropropane and shake samples by hand vigorously for 15 seconds. Incubate 10 minutes at room temperature to additionally improve the purity of the RNA.

Centrifuge the lysate at 12.000 g for 15 minutes at +4°C. During centrifugation the mixture separates into the lower red (phenol-chloroform phase), the interphase and the colourless upper aqueous phase. RNA is forced exclusively into the aqueous phase whereas DNA and the proteins partition into the interphase and lower phenol phase.

> **Note**: Chloroform used in this experiment should be free of additives like isoamyl alcohol or others.

1.3) RNA precipitation

Transfer the upper, aqueous phase to a new reaction tube and add the same volume of isopropanol to precipitate the RNA on ice for at least 15 minutes. Keep the organic phase/interphase with the DNA/protein at +4°C.

Centrifuge at 12.000 g for 15 minutes at +4°C. The RNA pellet should form a gel like precipitate on the bottom and on the side of the tube.

1.4) RNA wash

Remove the supernatant carefully and wash the RNA pellet with ethanol (70-80%) by vortexing and subsequently with absolute ethanol (100%).

Centrifuge at 7500 g at +4°C or room temperature for 8 minutes.

1.5) RNA solubilization

Remove the excess ethanol from the RNA pellet by air-drying the sample.

Note: Do not let the RNA pellet dry completely as a dry pellet will be much less soluble.

Dissolve in 20 µl DEPC-treated water. Dissolve the RNA pellet by passing the solution through a pipette tip several times.

2.) Method for DNA isolation

During the phase separation step DNA is forced out of the aqueous phase. After precipitation from the organic phase and a few washes the DNA is dissolved 1X TE buffer. This DNA isolated by TriFast II can be used for PCR, Southern blots and restriction enzyme digests.

Reagents required, but not supplied

- Ethanol
- Sodium citrate
- TE buffer

2.1) DNA precipitation

Remove any left aqueous phase after RNA removal (step 1.3); this will improve the purity of the DNA dramatically.

Add 0.3 ml of 100% ethanol per ml TriFast II originally employed to selectively precipitate the DNA.

Mix well by inversion and incubate the mixture for approx. 5 minutes at room temperature.

Centrifuge at 2.000 g for 5 minutes at +4°C.

Remove the supernatant (phenol/ethanol mix) and keep the supernatant at +4°C for the subsequent protein purification.

2.2) DNA wash

Wash the DNA precipitate in 1 ml 0,1 M sodium citrate/10 % ethanol (per 1 ml TriFast II reagent used in the original homogenization). Incubate for 30 minutes at room temperature; mix from time to time.

Centrifuge at 2.000 g for 5 minutes at room temperature.

Repeat the wash step.

2.3) DNA solubilization

Air-dry the DNA and dissolve in approx. 0,5 ml 1X TE (10 mM Tris, 1 mM EDTA; pH 8,0). Remaining cell fragments in this mixture are sedimented by centrifugation (12.000 g/10 minutes), while the DNA stays in solution under these conditions.

Transfer the DNA-containing supernatant to a new reaction tube.

> **Note**: High viscosity at this point indicates high molecular weight DNA.

3.) Method for protein isolation

Proteins can be isolated from the phenol/ethanol supernatant obtained after DNA precipitation (step 2.1 of DNA Precipitation). This solution can be further analyzed for the presence of specific proteins i.e. by Western blotting.

Reagents required, but not supplied

- Isopropanol
- Guanidine hydrochloride
- Ethanol
- SDS

3.1) Protein precipitation

Add the double sample volume of isopropanol to the phenol/ethanol mixture from step 2.1. Proteins will be precipitated during an incubation of approx. 10 minutes at room temperature.

Centrifuge at 12.000 g for 10 minutes at +4°C.

3.2) Protein wash

Wash the protein precipitate with 2 ml of 300 mM guanidine hydrochloride in 95 % ethanol per 1 ml Trifast II reagent used for the initial homogenization. Incubate for 20 minutes at room temperature.

Centrifuge at 7.500 g for 5 minutes at +4°C.

Repeat twice the wash step.

3.3) Protein solubilization

Remove the supernatant and Air-dry or vacuum-dry the protein precipitate.

Dissolve the precipitate in 1 % SDS. Warming up to 50°C might be necessary.

> **Note**: If you detect residual cell fragments or other insoluble components, remove them by an additional centrifugation step (10.000 g/10 minutes/+4°C).

Transfer the supernatant to a new reaction tube (storage -20°C).

Troubleshooting

Problem	Likely cause
RNA isolation	**Troubleshooting**
Low yield	• Incomplete homogenization or incomplete lysis. • Final RNA pellet not completely dissolved.
A260/280 ratio < 1.65	• Sample homogenized in too small volume of reagent. • After homogenization samples were not stored at room temperature for 5 minutes • The aqueous phase was contaminated with the phenol phase. • Incomplete dissolution of thefinal RNA pellet.
RNA degradation	• Tissues were not immediately processed or frozen. • RNAse contamination was introduced during preparation. • Samples used for isolation were frozen at -20°C instead of -70 °C.
DNA contamination	• Sample homogenized in too small volume of reagent. • Samples used for the isolation contained organic solvents (ethanol, DMSO, strong buffers or alkaline pH).
DNA isolation	
Low yield	• Incomplete homogenization or incomplete lysis. • Final DNA pellet not completely dissolved.
A260/280 ratio < 1.65	Phenol was not sufficiently removed from the DNA preparation. Wash the DNA pellet with 0.1 M sodium citrate in 10% ethanol.
DNA degradation	• Tissues were not immediately processed or frozen. • Samples used for isolation were frozen at -20°C instead of -70 °C. • Samples were homogenized with too high speed resulting in shearing of the DNA.
RNA contamination	• Aqueous phase was incompletely removed. • DNA pellet was not sufficiently washed.
Protein isolation	
Low yield	• Incomplete homogenization or incomplete lysis. • Final Protein pellet not completely dissolved. • Protein degradation. • Tissues were not immediately processed or frozen.
Band deformation in PAGE	Protein pellet insufficiently washed

Caution: TriFast II contains Phenol and Guanidinium thiocyanate.

Safety precautions: Harmful if swallowed. Toxic in contact with skin. Toxic if inhaled. Causes severe skin burns and eye damage. Causes serious eye damage. Suspected of causing genetic defects. May cause damage to organs through prolonged or repeated exposure. Harmful to aquatic life with long lasting effects. Please refer to the material safety data sheet for information regarding hazards and safe handling practice

Signal word: DANGER

EMR517100_EMR517200_TriFast

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