TriFast™

Isolation of RNA, DNA and Protein simultaneously

Cat.No.	**30-2010**	**100ml**
	30-2020	**200ml**
	30-2030	**500ml**

Storage: **Store at 4°C**. Protect from long exposures (days) to light.

Description

TriFast™ is a complete ready to use reagent for the simultaneous isolation of RNA, DNA and proteins from 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™). This method has been shown to yield undegraded RNA from small and large samples. Also it makes possible to process a large number of samples simultaneously.

TriFast™ includes phenol and guanidinium thiocyanate in a monophasic solution. After addition of chloroform the homogenate separates into three phases upon centrifugation. The aqueous phase containing the RNA, the interphase the DNA and the organic phase the protein 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.

TriFast™ is stable for 12 months from the date of purchase when stored at 4°C.

WARNING: TriFast™ contains phenol and guanidinium thiocyanate. This combination can be fatal. During work with TriFast™ gloves and eye protection should be used. In case of contact with skin or eyes etc. wash excessively with water for at least 15 minutes and seek medical care. Work in a hood.

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A. Instruction for RNA Isolation

The RNA isolation procedure can be completed in 1 hour. The yield of RNA is comparable or higher than with any other method. The total RNA isolated by TriFast™ 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 included

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

The procedure includes the following steps:

1. Homogenization	- 1.0 ml TriFast™ + 50-100 mg sample
2. Phase Separation	- homogenate + 0.2 ml chloroform
3. RNA Precipitation	- aqueous phase + 0.5 ml isopropanol
4. RNA Wash	- 1 ml 75% ethanol
5. RNA Solubilization	- Formamid, 0.5% SDS, or water

The procedure includes the following steps and is carried out at room temperature if not stated otherwise.

1. Homogenization

a. Tissues

Homogenize tissue samples in 1ml TriFast™ 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™ used for the homogenization.

b. Cells grown in Monolayer

Lyse cells directly in a culture dish by addition of 1 ml TriFast™ to a 3.5 cm diameter dish and passing the cell lysate several times through a pipette. The amount of TriFast™ 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™ may result in contamination of the isolated RNA with DNA.

c. Cells grown in suspension

Pellet celly by centrifugation. Lyse cells in TriFast™ by pipetting. Add 1 ml reagent per 5-10 x 10^6 of animal, plant, yeast or bacterial cells. Washing cells before the addition of TriFast™ should be avoided as this increases the possibility of RNA degradation. Disruption of some yeast or bacterial cells may require the use of a homogenizer.

2. Phase Separation

For dissociation of the nucleoprotein complexes the samples should be kept for 5 minutes at room temperature. Shake samples by hand vigorously after addition of 0.2 ml of chloroform for 15 seconds and keep them for 3-10 minutes at room temperature.
During centrifugation at 12.000 x g (max) 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. The volume of the RNA containing phase is about 60% of the volume of the TriFast™ used for homogenization.
Chloroform used in this experiment should be free of additives like isoamyl alcohol or others.

3. RNA Precipitation

Transfer the aqueous phase to a fresh appropriate tube (save the interphase and organic phase at 4°C for the isolation of DNA and proteins). Precipitate the RNA with 0.5 ml of Isopropanol per 1 ml of TriFast™ used for the initial homogenization. Keep samples on ice for 5-15 minutes and centrifuge for 10 minutes at 4°C at 12.000 g max. The RNA pellet should form a gel like precipitate on the bottom and on the side of the tube.

4. RNA Wash

Remove the supernatant carefully and wash the RNA pellet twice with 75% ethanol by vortexing and subsequent centrifugation for 8 minutes at 7,500 x g (4°C).

5. RNA Solubilization

Remove the excess isopropanol from the RNA pellet by air-drying or placing the sample under vacuum for 5–10 minutes.
Caution: Do not dry the RNA pellet by centrifugation under vacuum. Do not let the RNA pellet dry completely as a dry pellet will be much less soluble.
Resuspend the RNA pellet in deionized formamide, Rnase-free water or in 0.5% SDS solution. Dissolve the RNA pellet by by passing the solution through a pipette tip several times, then incubating the solution for 10 -15 minutes at 55°C to 60°C.
pipetting it up and down. Heating the sample at 55-60°C might help to dissolve the pellet.

TriFast™ isolates the whole range of RNAs including mRNA and rRNA. The final preparation of total RNA is free of DNA and proteins and has a 260/280 ratio of 1.6- 2.0.

Notes and Comments

1. Hands and dust in the laboratory can be the major source for RNase contaminations. Use gloves and keep the tubes closed during a RNA preparation.
2. When small amounts of RNA are expected (<10 mg) homogenization should be performed in the presence of 70 mg of glycogen carrier (Cat.No.A 1030 S) per 1 ml **TriFast**™ used.
3. 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 4 can be stored in 75% ethanol for 1-3 weeks at 4°C or for at least one year at -20°C.
4. 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 and 3 of the DNA isolation protocol.

Troubleshooting Guide RNA Isolation

Expected yields of RNA per mg of tissue or 10^6 cells

Liver and spleen	6-10 µg
Kidney	3-4 µg
Skeletal muscles and brain	1-1.5 µg
Placenta	1-4 µg
Epithelial cells	8-15 µg
Fibroblasts	5-7 µg

Low yield

- Incomplete homogenization or incomplete lysis
- Final RNA pellet not completely dissolved.

A260/A280 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 the final RNA pellet.

RNA degradation

- Tissues were not immediately processed or frozen.
- Cells were dispersed by Trypsin.
- RNAse contamination was introduced during preparation.
- Samples used for isolation were frozen at -20°C instead of -70 °C.
- Formaldehyde solution used for electrophoresis had a pH below 3.5.

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).

B. Instruction 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 in 8 mM NaOH and neutralized. This DNA isolated by **TriFast**™ can be used for PCR, Southern blots and restriction enzyme digests.

Reagents needed but not supplied
- Ethanol
- Sodium citrate
- Sodium hydroxide

The procedure includes the following steps and is carried out at room temperature if not stated otherwise.

1. DNA Precipitation - phenol and interphase + 0.3 ml ethanol starting from 1 ml **TriFast**™
2. DNA Wash - 1 ml 0.1M sodium citrate in 10% ethanol - 2 ml ethanol 75%
3. DNA Solubilization - 8 mM NaOH

1. DNA Precipitation

Remove any left aqueous phase after RNA removal and precipitate the DNA with 0.3 ml of 100% ethanol per ml TriFast™. Mix well by inversion and store the samples at room temperature for 2-3- minutes. DNA is sedimented by centrifugation at 2,000 x g at 4°C for 5 minutes. (Careful removal of the aqueous phase is critical for the quality of the isolated DNA)

2. DNA Wash

Remove the DNA supernatant (ethanol/phenol mix) and store it at 4°C for the protein isolation. The DNA pellet is washed twice with 1 ml 0.1 M sodium citrate in 10% ethanol. At each wash step keep the DNA in 0.1 M sodium citrate / 10% ethanol for 30 minutes at room temperature (with periodic mixing), then centrifuge at 4°C for 5 minutes at 2,000 x g.
After these two washes suspend the DNA pellet in 2 ml of 75% ethanol, keep it at room temperature with periodic mixing for 15 minutes and centrifuge at 2,000 x g for 5 minutes at 4°C.
A third washing step is recommended for amounts >200 µg of DNA.

3. DNA Solubilization

Dry the DNA pellet briefly for 5-10 minutes under vacuum and dissolve it in 8 mM NaOH by slowly passing the pellet through a wide bore pipet. Try to adjust the final concentration of DNA to 0.2-0.3 µg/µl with 8 mM NaOH. For 50-70 mg of tissue or 10^7 cells about 0.3-0.6 ml of 8 mM NaOH are needed. This procedure will solubilize the DNA safely. Nevertheless the DNA preparation often contains gel-like materials. Remove these materials by centrifugation at 12,000 x g for 10 minutes.
Transfer the DNA to a fresh tube. High viscosity at this point indicates high molecular weight DNA. For neutralization of the DNA solution please use the data in the table on page 6.

Quantization of DNA

Take an aliquot of the DNA preparation and dilute it with water to yield an appropriate optical density at 260 nm.

- Assume that 1 A_{260} unit corresponds to 50 µg of double-stranded DNA
- Assume that the theoretical amount of DNA per 10^6 diploid cells of human, rat and mouse equals 7.1 mg, 6.5 mg and 5.8 mg respectively. Typically the final DNA from tissues should have a size distribution of 50-100 kb (75%) and about 20 kb (25%), DNA from cells 50-100 kb (80%) and 20% with a size of 20 kb. The DNA should be free of RNA and proteins with $A_{260/280}$ ratio of >1.7.

Applications

2. Amplification of DNA by PCR
   After redissolving the DNA in 8 mM NaOH the pH is adjusted to 8.4 with 0.1 M HEPES (see table). Use between 0.1 and 1.0 mg of the DNA sample for a PCR reaction.
2. Restriction enzyme digests
   Adjust the pH to an appropriate value using HEPES (see table). Add the buffer system recommended by the enzyme supplier and proceed as usual.
3. pH adjustment table of DNA samples dissolved in 8 mM NaOH (for 1 ml of 8 mM NaOH add the indicated amounts of 0.1 M or 1 M HEPES-free acid)

Final pH	0.1 M HEPES (µl)	Final pH	1 M HEPES (µl)
8.4	66	7.2	30
8.2	90	7.0	42
8.0	115		
7.8	135		
7.5	180		

Notes

1. The phenol and interphase can be stored at 4°C overnight.
2. Samples in 75% ethanol can be stored at 4°C for months.
3. Samples in 8 mM NaOH can be stored overnight at 4°C. For long-term storage adjust the pH to 8.0 and add EDTA to a final concentration of 1 mM. Do not freeze the DNA.

Troubleshooting Guide DNA Isolation

Expected yields of DNA per mg of tissue or 10^6 cells

Liver and kidney	3-4 µg
Skeletal muscles, brain, and placenta	2-3 µg
Cultured human, rat and mouse cells, Fibroblasts	5-7 µg

Low yield

- Incomplete homogenization or incomplete lysis. Final DNA pellet not completely dissolved.

$A_{260/280}$ 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.

C. Instructions for Protein Isolation

Proteins can be isolated from the phenol/ethanol supernatant obtained after DNA precipitation (see step 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

- SDS
- Guanidine hydrochloride
- Ethanol
- Isopropanol.

The procedure includes the following steps and is carried out at room temperature if not stated otherwise.

1. Protein Precipitation	phenol/ethanol supernatant + 1.5 ml Isopropanol
2. Protein Wash	2 ml of 0.3 M guanidine hydrochloride in 95% ethanol, 3 x 20 minutes each. 1 x ethanol(100%) wash for 20 minutes
3. Protein solubilization	1% SDS

1. Protein Precipitation

Precipitate the proteins from the ethanol/phenol supernate (approx. 0.8 ml) with 1.5 ml Isopropanol. Store the samples at room temperature for at least 10 minutes and sediment the protein precipitate at 12.000 g for 10 minutes at 4°C.

2. Protein Wash

Remove supernatant and wash the protein pellet 3 times with a 2 ml solution of 0.3 M guanidinium hydrochloride in 95% ethanol- keep the samples in washing solution for 20 minutes at room temperature before centrifuging at 7.500 g for 5 minutes at 4°C). Next vortex the protein pellet once with 2 ml of 100% ethanol, store for 20 minutes at room temperature and centrifuge at 7.500 g for 5 minutes at 4°C.

3. Protein solubilization

Dry the Protein pellet briefly for 5-10 minutes under vacuum and dissolve it in 1% SDS by pipetting it up and down. Incubation of the samples at higher temperatures (50-100°C) may be necessary to yield complete solubilization. Unsoluble material should be removed by centrifugation at 10.000 g for 10 minutes at 4°C. The protein supernatant should be transferred to a fresh tube. It can either be used immediately or kept frozen at -20°C for future use.

Notes

1. The critical step in the protein isolation is the solubilization step. The following alternative may yield better results. Dialyze the phenol/ethanol supernatant against three changes of 0.1% SDS at 4°C. Then centrifuge the dialyzate at 10.000 g for 10 minutes at 4°C and use the clear supernatant for Western blotting or freeze it at -20 °C.
2. The protein suspended either in 0.3 M guanidine hydrochlorid-95% ethanol or in ethanol is stable for one month at 4°C or more than one year at -20 °C.
3. Proteins can be quantified according to the Bradford method if the SDS concentration is lower than 0.1%.

Trouble Shooting Guide Protein Isolation

Low yield

- Incomplete homogenization or incomplete lysis.
- Final Protein pellet not completely dissolved.
- Protein degradation.
- Tissues were not immidiately processed or frozen.

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- Protein pellet insufficiently washed