For life science research only.
Not for use in diagnostic procedures.

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TriPure Isolation Reagent

Version 08

Content version: March 2018

Monophasic solution of phenol and guanidine thiocyanate for RNA, DNA, and protein isolation

Cat. No. 11 667 157 001	50 ml
Cat. No. 11 667 165 001	200 ml

Store the kit at +2 to +25°C

sigma-aldrich.com

Table of Contents

1. What this Product Does

Contents

Cat. No.	Content
11 667 157 001	50 ml
11 667 165 001	200 ml

Form

TriPure Isolation Reagent is a clear, red, monophasic solution of phenol and guanidine thiocyanate. It is ready to use as supplied.

Storage and Stability

- The reagent is stable through the control date printed on the label (24 months from the date of manufacture) when stored unopened at +2 to +25°C in the original container.
- Do not expose to light for long periods (days).
- TriPure Isolation Reagent is shipped in a polypropylene bottle at ambient temperature.

Application

TriPure Isolation Reagent allows the isolation of total RNA, DNA, and protein from the same sample in a single-step liquid-phase separation (1). The procedure is an improvement of the single-step RNA isolation method developed by Chomczynski and Sacchi (2). This reagent performs well with both small and large quantities of tissue or cells. The cells may be of human, animal, plant, or bacterial origin. RNA is undegraded and free of protein or DNA contamination.

2. How to Use this Product

2.1 Before you Begin

Precautions

- TriPure Isolation Reagent contains phenol (a poison) and guanidine thiocyanate (an irritant). This reagent is toxic. This reagent can also cause burns if it touches the skin.
- Do not allow guanidine thiocyanate to contact sodium hypochlorite (bleach) solution or acids. These mixtures produce a highly toxic gas.
- When using TriPure Isolation Reagent, work under a fume hood. Wear gloves, lab coat, and appropriate eye protection (shield, safety goggles). Avoid breathing vapor.
- **IF YOU GET THE REAGENT ON YOUR SKIN,** wash skin immediately with soap or mild detergent, and flush with large amounts of water for 15 to 30 min, until no evidence of chemical remains.
- **IF YOU GET THE REAGENT IN YOUR EYES,** flush eyes with large amounts of water for 15 to 30 min, until no evidence of chemical remains.

2.2 Materials and Reagents Required

For Extraction and Phase Separation

- Sterile, disposable polypropylene tubes that can withstand 12,000 × *g* in the presence of TriPure Isolation Reagent and chloroform
- Homogenization apparatus (for tissue and certain cells only
- Reagents for density gradients (for white blood cells only)
- Chloroform (free of all additives such as isoamyl alcohol)
- Glycogen (for tissue samples <10 mg)

For RNA Isolation

- Isopropanol
- 75% Ethanol
- Diethylpyrocarbonate (DEPC)-treated RNase-free water or DEPC-treated 0.5% SDS

For DNA Isolation

- 100% Ethanol
- 8 mM NaOH
- 0.1 M Sodium citrate in 10% ethanol

For Protein Isolation

- Isopropanol
- 100% Ethanol
- 1% SDS
- 0.3 M Guanidine hydrochloride in 95% ethanol

2.3 Isolation Procedures

Summary

During sample homogenization or lysis, the TriPure Isolation Reagent disrupts cells and denatures endogenous nucleases, thus preserving the integrity of RNA and DNA in the sample. After chloroform is added to the extract, the entire mix is centrifuged. After centrifugation, the solution contains three phases — a colorless aqueous (upper) phase, a white interphase, and a red organic (lower) phase.

The upper phase is placed in a separate tube from the other two phases. RNA is recovered from the colorless aqueous phase by isopropanol precipitation. DNA and protein are sequentially isolated from the white interphase and the red organic phase by alcohol precipitation steps.

Expected Yield of RNA

- **Tissues:** liver or spleen, 6 to 10 μg/mg tissue; kidney, 3 to 4 μg/mg tissue; skeletal muscle or brain, 1 to 1.5 μg/mg tissue; placenta, 1 to 4 μg/mg tissue
- **Cultured cells:** epithelial cells, 8 to 15 $\mu g/10^6$ cells; fibroblasts, 5 to 7 $\mu g/10^6$ cells.

Expected Yield of DNA

- **Tissues:** liver or kidney, 3 to 4 μg/mg tissue; skeletal muscle, brain, or placenta, 2 to 3 μg/mg tissue.
- **Cultured cells:** human, rat, or mouse cells, 5 to 7 $\mu g/10^6$ cells.

Precautions for Preventions of RNase Contamination

- Wear clean disposable gloves at all times
- Use sterile disposable plasticware and pipettes/tips reserved for RNA work only.
- For more information on maintaining an RNase-free environment, see reference 4.

2.4 Procedure 1

Extraction of RNA, DNA, and Protein Form Tissues or Cells

Keep track of the amount of TriPure Isolation Reagent that you use for each sample in this procedure. The amount of TriPure used will determine the amount of reagents required in later procedures

⚠ TriPure Isolation Reagent is a hazardous reagent. When using it, observe all safety precautions listed in chapter "Precautions".

2.4.1 If You Start with Tissue

1. For each 50 to 100 mg of tissue to be processed, add 1 ml TriPure Isolation Reagent to a polypropylene centrifuge tube at +15 to +25°C. The capacity of the tube should be at least twice the volume of the tissue and TriPure Isolation Reagent combined.

   For small (<10 mg) tissue samples, use 0.8 ml TriPure Isolation Reagent plus 5 to 10 µg glycogen (as a carrier to assist RNA precipitation).

   ⚠ The volume of the sample should not be greater than 10% of the volume of TriPure Isolation Reagent.

2. Add fresh or frozen tissue samples to the tube.

3. Homogenize the tissue with a glass-Teflon or power homogenizer (Polytron or equivalent).

   The molecular weight of DNA isolated in Procedure 4 is dependent on the shearing forces applied during homogenization. Use a loosely fitting homogenizer, and avoid use of a Polytron homogenizer if you want high molecular weight DNA. The use of a hand-held homogenizer may decrease overall yields but will minimize shearing of high molecular weight DNA.

4. (Optional step, necessary only for tissues that contain large amounts of proteins, fat, polysaccharides, or extracellular material [*e.g.*, muscle tissue, fatty tissue, or tuberous parts of plants]). Clarify the homogenate by centrifuging it at 12,000 × *g* for 10 min at +2 to +8°C. Extracellular membranes, polysaccharides, and high molecular weight DNA pellet during the centrifugation, while excess fat collects as a layer on top of the supernatant. Remove the fatty layer (if any), then transfer the supernatant (which contains the RNA, most DNA, and protein) to a fresh polypropylene centrifuge tube.

2.4.2 If You Start with Adherent Cells Grown in a Monolayer

Option A

1. Decant culture medium from cells.
2. At +15 to +25°C, add directly to the culture dish or flask, 1 ml TriPure Isolation Reagent for each 10 cm^2 area covered by cells (regardless of cell number).

   Adding too little TriPure Isolation Reagent may lead to contamination of isolated RNA with DNA.
3. Pass the cell lysate through a pipette several times.
4. Transfer the cell lysate to a polypropylene centrifuge tube. The capacity of the tube should be at least twice the volume of the cells and TriPure Isolation Reagent combined.

Option B

1. Decant culture medium from cells.
2. At +15 to +25°C, add directly to the culture dish or flask, 3 ml TriPure Isolation Reagent for each 5 to 10×10^6 cells.
3. Scrape cells from the walls of the flask or dish into the reagent to lyse them.
4. Pass the cell lysate through a pipette several times.
5. Transfer the cell lysate to a polypropylene centrifuge tube. The capacity of the tube should be at least twice the volume of the cells and TriPure Isolation Reagent combined.

2.4.3 If You Start with Cells Grown in Suspension

1. Pellet cells by centrifugation in a polypropylene centrifuge tube. The capacity of the tube should be at least twice the volume of the pelleted cells and TriPure Isolation Reagent combined. Remove the supernatant.

   Do not wash cells before adding TriPure Isolation Reagent.
2. Add TriPure Isolation Reagent directly to the tube containing the cell pellet at +15 to +25°C. Use 1 ml reagent for each 5 to 10×10^6 animal, plant, or yeast cells; or 1 ml reagent for each 1×10^7 bacterial cells.

   For small amounts ($< 10^6$) of cells, use 0.8 ml TriPure Isolation Reagent plus 5 to 10 µg glycogen (as a carrier to assist RNA precipitation).
3. Lyse cells by repetitive pipetting or homogenization.

   The molecular weight of the isolated DNA is dependent on the shearing forces applied during homogenization. Use a loosely fitting homogenizer and avoid use of a Polytron homogenizer if you want high molecular weight DNA.
4. Transfer the cell lysate to a polypropylene centrifuge tube.

2.4.4 If You Start with Human Blood

❶	Isolate buffy coat or lymphocytes from human whole blood using low-speed centrifugation or density gradients
❷	For each 5 to 10 × 10^6 white blood cells, add 1 ml TriPure Isolation Reagent directly to the centrifuge tube
❸	Lyse cells by repetitive pipetting or homogenization

Molecular weight of the isolated DNA is dependent on the shearing forces applied during homogenization. Use a loosely fitting homogenizer, and avoid use of a Polytron homogenizer if you want high molecular weight DNA.

2.5 Procedure 2

Phase Separation

The sample homogenates obtained in Procedure I (Extraction) can be stored at –60°C or below for at least 1 month before they are used in Procedure 2 (Phase separation).

❶	Incubate each homogenized sample from Procedure I for 5 min at +15 to +25°C to ensure the complete dissociation of nucleoprotein complexes.
❷	Add chloroform to each sample. Use 0.2 ml chloroform for each 1 ml TriPure Isolation Reagent required in the initial homogenization (Procedure I).
❸	Cap tube securely, and shake it vigorously for 15 sec.
❹	Incubate tube at +15 to +25°C for 2 to 15 min.
❺	To separate the solution into three phases, centrifuge tube at 12,000 × *g* for 15 min at +2 to +8°C ⚠ Do not exceed 12,000 × *g* during centrifugation.
❻	After centrifugation, use the three phases of each solution as follows • From the colorless upper aqueous phase (which is approximately 60% of the volume of TriPure Isolation Reagent used in Procedure 1), isolate RNA according to Procedure 3. • From the interphase and lower red organic phase, isolate DNA (according to Procedure 4) and protein (according to Procedure 5).

2.6 Procedure 3

Isolation of RNA

1. Transfer the colorless upper aqueous phase obtained in Procedure 2 to a new polypropylene centrifuge tube.

   Save the interphase and lower red organic phase obtained in Procedure 2 from each sample, and use these phases to isolate DNA (according to Procedure 4) and protein (according to Procedure 5).

2. Precipitate the RNA from the colorless aqueous phase of each sample by performing the following steps:
   a. Add isopropanol to the aqueous phase. Use 0.5 ml isopropanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization (Procedure I).
   b. Cap the tube, then invert it several times to mix it thoroughly.
   c. Incubate sample for 5 to 10 min at +15 to +25°C to allow the RNA precipitate to form.
   d. Centrifuge the sample at 12,000 × *g* for 10 min at +2 to +8°C.
   e. Discard the supernatant.

3. Add 75% ethanol to each centrifuge tube. Use at least 1 ml of 75% ethanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization procedure.

   The RNA precipitate can be stored in 75% ethanol for at least 1 week at +2 to +8°C, or at least 1 year at −15 to −25°C.

4. Wash the RNA pellet in the ethanol by vortexing.

5. Centrifuge the sample at 7,500 × *g* for 5 min at +2 to +8°C. Discard the supernatant.

6. Remove the excess ethanol from the RNA pellet by air-drying or placing the sample under vacuum for 5 to 10 min.

   ⚠ 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.

7. Resuspend the RNA pellet in diethylpyrocarbonate (DEPC)-treated RNase-free water or DEPC-treated 0.5% SDS.

8. Dissolve the RNA pellet by passing the solution through a pipette tip several times, then incubating the solution for 10 to 15 min at +55°C to +60°C.

2.7 Procedure 4

Isolation of DNA

The interphase and red organic phase obtained in Procedure 2 can be stored at +2 to +8°C overnight before they are used in Procedure 4.

1. Carefully remove all the remaining colorless upper aqueous phase from each sample obtained in Procedure 2, and discard.

   ⚠ You must get rid of all the aqueous phase in order to obtain high quality DNA.

2. Precipitate the DNA from the interphase and red organic phase of each sample by performing the following steps:
   a. Add 100% ethanol to the interphase and organic phase. Use 0.3 ml 100% ethanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization (Procedure I).
   b. Cap the sample, then invert it several times to mix it thoroughly.
   c. Incubate sample for 2 to 3 min at +15 to +25°C to allow the DNA precipitate to form.
   d. Centrifuge the sample at 2,000 × *g* for 5 min at +2 to +8°C.

3. Remove the supernatant (containing phenol, ethanol, and protein) from each sample, and save at +2 to +8°C. Use this supernatant to isolate protein (according to Procedure 5).

4. To remove any phenol present in the DNA from each sample, perform the following steps:
   a. Add 0.1 M sodium citrate in 10% ethanol to the pellet remaining in the centrifuge tube. Use 1 ml sodium citrate/ethanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization procedure.
   b. Incubate the sample, with occasional mixing, for 30 min at +15 to +25°C.
   c. Centrifuge the sample at 2,000 × *g* for 5 min at +2 to +8°C.
   d. Discard the supernatant.

5. Repeat Step 4 twice (for a total of 3 sodium citrate/ethanol washes).

6. After the 3 sodium citrate/ethanol washes, perform the following steps for each DNA precipitate:
   a. Wash each DNA pellet in 75% ethanol. Use 1.5 to 2.0 ml ethanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization procedure.

      DNA suspended in 75% ethanol can be stored at +2 to +8°C for several months.

   b. Incubate sample, with occasional mixing, at +15 to +25°C for 10 to 20 min.
   c. Centrifuge the sample at 2,000 × *g* for 5 min at +2 to +8°C.
   d. Discard the supernatant.

❼ Remove the excess ethanol from each DNA pellet by air-drying or placing the sample under vacuum for 5 to 10 min.

❽ Dissolve the DNA pellet by performing the following steps:

a. To the pellet, add enough 8 mM NaOH to approach a DNA concentration of 0.2 to 0.3 µg/µl.
Example: Use 0.3 to 0.6 ml 8 mM NaOH to resuspend DNA isolated from 50 to 70 mg tissue or 10^7 cells.

b. Pass the sample through a pipette.

DNA will fully dissolve in the mild alkaline solution. However, the dissolved DNA (especially from tissue samples) may still contain insoluble gel-like material (fragments of membranes, *etc.*).

c. (optional) If the dissolved DNA contains insoluble material, pellet the insoluble material by centrifugation (12,000 × *g* for 10 min at +2 to +8°C), then transfer the clear supernatant (DNA) to a new tube before storing it.

❾ For long-term storage, adjust the pH of the DNA solution to pH 7 to 8 with HEPES, and adjust the EDTA concentration to 1 mM.

Samples solubilized in 8 mM NaOH can be stored overnight at +2 to +8°C without neutralization.

2.7.1 Guidelines for Using DNA Isolated with TriPure Isolation Reagent

Calculation of Cell Number

To calculate cell number from DNA content, assume the amount of DNA in 10^6 diploid cells of human, rat, and mouse origin equals 7.1 µg, 6.5 µg, and 5.8 µg, respectively (3).

Digestion of DNA by Restriction Endonucleases

Adjust the pH of the DNA solution isolated in Procedure 4 to the required value with HEPES according to Table 1. Alternatively, dialyze samples against 1 mM EDTA, pH 7.0 to 8.0. Digest the DNA for 3 to 24 hours, with 3 to 5 units of enzyme per µg DNA under conditions optimal for the specific restriction enzyme. In a typical restriction assay, 80 to 90% of the DNA preparation is digestible.

Amplification of DNA by PCR

Adjust the pH of the DNA isolated in Procedure 4 to 8.4 using 0.1 M HEPES (see Table 1). Add 0.1 to 1.0 µg of the DNA sample to a standard PCR.

Tab. 1: Amount of HEPES buffer required to adjust 1 ml of DNA from Procedure 4 (in 8 mM NaOH) to different pH values.

For a final pH of...	add this amount of 0.1 M HEPES (µl)	or this amount of 1.0 M HEPES (µl)
8.4	86	–
8.2	93	–
8.0	101	–
7.8	117	–
7.5	159	–
7.2	–	23
7.0	–	32

2.8 Procedure 5

Isolation of Protein

Isolate proteins from the phenol-ethanol supernatant obtained in Procedure 4, Step 3.

Normal Procedure

1. Precipitate proteins from the phenol-ethanol supernatant of each sample by performing the following steps:
   a. Add isopropanol to the phenol-ethanol supernatant. Use 1.5 ml isopropanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization (Procedure 1).
   b. Cap the tube, then invert it several times to mix it thoroughly.
   c. Incubate sample for a minimum of 10 min at +15 to +25°C to allow the protein precipitate to form.
   d. Centrifuge the sample at 12,000 × *g* for 5 min at +2 to +8°C.
   e. Discard the supernatant.
2. To wash the precipitated protein in each sample, perform the following steps:
   a. Resuspend each protein pellet with 0.3 M guanidine hydrochloride in 95% ethanol. Use 2 ml guanidine hydrochloride/ethanol for each 1 ml TriPure Isolation Reagent required in the initial homogenization procedure.
   The protein pellet suspended in 0.3 M guanidine hydrochloride/95% ethanol can be stored for at least 1 month at +2 to +8°C or for at least one year at –15 to –25°C.
   b. Mix the sample, and incubate at +15 to +25°C for 20 min.
   c. Centrifuge the sample at 7,500 × *g* for 5 min at +2 to +8°C.
   d. Discard the supernatant.
3. Repeat Step 2 twice (for a total of 3 guanidine hydrochloride/ethanol washes).
4. After the 3 washes, perform the following steps on each protein pellet:
   a. Add 2 ml 100% ethanol to each protein pellet.
   b. Vortex to wash the pellet.
   c. Incubate at +15 to +25°C for 20 min.
   d. Centrifuge at 7,500 × *g* for 5 min at +2 to +8°C.
   e. Discard supernatant.
5. Remove the excess ethanol from each protein pellet by air-drying or placing the sample under vacuum for 5 to 10 min.
6. Dissolve the protein pellet by adding 1% SDS to it and repeatedly passing the solution through a pipette.
   You may need to incubate the SDS-pellet mixture at +50°C to completely solubilize the protein.

7. Sediment any insoluble material by centrifuging the resuspended protein at 10,000 × *g* for 10 min at +2 to +8°C.
8. Transfer the supernatant containing the protein to a new tube.

   The sample may be used immediately or may be stored at −15 to −25°C. The protein solution may be analyzed directly on a western blot.

Alternate Procedure

To recover proteins more efficiently, perform the following steps:

1. Dialyze the phenol-ethanol supernatant against 3 changes of 0.1% SDS at +2 to +8°C.
2. Centrifuge the dialyzed material at 10,000 × *g* for 10 min at +2 to +8°C.
6. Transfer the supernatant containing the protein to a new tube.

   The sample may be used immediately or may be stored at −15 to −25°C. The protein solution may be analyzed directly on a western blot.

3. Results

RNA Total RNA isolated with TriPure Isolation Reagent is undegraded and free of protein and DNA. The isolated RNA has an A_{260}/A_{280} ratio of 1.6 to 2.0. It is suitable for northern blot analysis, poly(A)$^+$ selection, in vitro translation, RNase protection assays, cloning, or RT-PCR.

DNA DNA isolated with TriPure Isolation Reagent is free of RNA and proteins and has an A_{260}/A_{280} ratio of >1.7. It is suitable for PCR, restriction digests, or Southern blots.

Protein Protein isolated with TriPure Isolation Reagent is suitable for western blots.

4. Troubleshooting

Procedure	Symptom	Probable Cause
RNA isolation	Low yield A_{260}/A_{280} ratio <1.65	• Incomplete sample homogenization or lysis. • Incomplete solubilization of final RNA pellet.
		• Too little TriPure Isolation Reagent used for sample homogenization. • Following homogenization, sample was not stored for 5 min at +15 to +25°C. • Contamination of the aqueous phase with phenol phase. • Incomplete solubilization of final RNA pellet.
	RNA degradation	• Tissues were not immediately processed or frozen after removal from animal. • Samples used for the isolation procedure were stored at −15 to −25°C instead of at −60°C or below. • Cells were dispersed by trypsin digestion. • Aqueous solutions or tubes not RNase-free.
	DNA contamination	• Too little TriPure Isolation Reagent used for sample homogenization. • Sample used for the procedure contained organic solvents (such as ethanol, DMSO) or strong buffers, or had an alkaline pH.
DNA isolation	Low yield	• Incomplete sample homogenization or lysis. • Incomplete solubilization of final DNA pellet.
	A_{260}/A_{280} ratio <1.7	• Incomplete removal of phenol from the DNA preparation (with sodium citrate/ethanol).
	DNA degradation	• Tissues were not immediately processed or frozen after removal from animal. • Sample used for the isolation procedure was stored at −15 to −25°C instead of at −60°C or below. • Sample was homogenized with a Polytron or other high speed homogenizer.
	RNA contamination	• Too much aqueous phase remained with the interphase and organic phase during Procedure 4. • Inadequate wash of the DNA pellet with 10% ethanol - 0.1 M sodium citrate solution.
Protein isolation	Low yield	• Incomplete sample homogenization or lysis. • Incomplete solubilization of final protein pellet.
	Protein degradation	• Tissues were not immediately processed or frozen after removal from animal.
	Band deformation in PAGE	• Insufficient wash of the protein pellet.

5. Additional Information on this Product

RNA

Total RNA isolated with TriPure Isolation Reagent is undegraded and free of protein and DNA. The isolated RNA has an A_{260}/A_{280} ratio of 1.6 to 2.0. It is suitable for northern blot analysis, poly(A)$^+$ selection, *in vitro* translation, RNase protection assays, cloning, or RT-PCR.

DNA

DNA isolated with TriPure Isolation Reagent is free of RNA and proteins and has an A_{260}/A_{280} ratio of >1.7. It is suitable for PCR, restriction digests, or Southern blots.

Protein

Protein isolated with TriPure Isolation Reagent is suitable for western blots.

References

1 Chomczynski, P. (1993) *BioTechniques* **15**, 532-537.

2 Chomczynski, P. & Sacchi, N. (1987) *Anal Biochem* **162**, 156-159.

3 Ausubel, F.M. *et al.* (1990) In: *Current Protocols in Molecular Biology*. New York: Wiley-Interscience. Vol. 2, Appendix 1, p. A.1.5.

4 Blumberg, D.D. (1987) *Methods Enzymol*. **152**, 20.

6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered stages labeled ①, ②, *etc.*	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

Editorial Changes.

6.3 Trademarks

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