RNA, DNA and protein isolation using TRIzol reagent

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Abstract: TRIZOL Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi. During sample homogenization or lysis, TRIZOL® Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropyl alcohol yields proteins from the organic phase. Beside the RNA isolation using TRIzol, TRIzol also can be used to isolate DNA and protein after the RNA isolation. This review and technical article just give a description of the TRIzol usage to help the reader to TRIzol reagent better. [Nature and Science. 2008;6(3):66-75]. ISSN: 1545-0740.

Keywords: RNA; TRIzol; isolation; gene; DNA

Introduction

RNA isolation and purification is one of the key factors for the RT-PCR assays and other related molecular biology detections. TRIzol Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (Gauthier, Madison and Michel, 1997). During sample homogenization or lysis, TRIZOL® Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropyl alcohol yields proteins from the organic phase (Chomczynski, 1993).

Beside the RNA isolation using TRIzol, TRIzol also can be used to isolate DNA and protein after the RNA isolation. TRIzol is a very useful reagent in RNA, DNA and protein isolation. This review and technical article just give a description of the TRIzol usage to help the readers to TRIzol reagent better, and to improve the development of the molecular biology researches.

Brief prescriptions

TRIZOL® Reagent, Cat. No. 15596-018 by Invitrogent Company, can be stored at 4-23^{o}C up to 12 months.

Copurification of the DNA may be useful for normalizing RNA yields from sample to sample (Eichler and Eales, 1985). This technique performs well with small quantities of tissue (50-100 mg) and cells (5 × 106), and large quantities of tissue (≥1 g) and cells (>107), of human, animal, plant, or bacterial origin. The simplicity of the TRIZOL® Reagent method allows simultaneous processing of a large number of samples (Ahmann et al., 2008). The entire procedure can be completed in one hour. Total RNA isolated by TRIZOL® Reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly (A)+ selection, in vitro translation, RNase protection assay, and molecular cloning. For use in the polymerase chain reaction (PCR*), treatment of the isolated RNA with amplification grade DNase I (Cat. No. 18068) is recommended when the two primers lie within a single exon.

TRIZOL® Reagent facilitates isolation of a variety of RNA species of large or small molecular size (Ahmann et al., 2008). For example, RNA isolated from rat liver, electrophoresed on an agarose gel, and stained with ethidium bromide, shows discrete bands of high molecular weight RNA between 7 kb and 15 kb in size, (composed of mRNA's and hnRNA's) two predominant ribosomal RNA bands at ~5 kb (28S) and at ~2 kb (18S), and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). The isolated RNA has an A260/A280 ratio ≥1.8 when diluted into TE.

Brief steps for the TRIzol usage on RNA isolation:

1. Add Trizol TRIzol platelet pellet (5-10 x 10^6 cells/1 ml).
2. Incubate at room temperature for 5 min.
3. Add 0.2 ml chloroform/1 ml TRIzol.
4. Shake tubes vigorously by hand for 15 seconds.
5. Incubate at room tempearture for 3 min.
6. Centrifuge 15 min at 4^{o}C at less than 12,000 g (10000 rpm, r=65 mm).
7. RNA is in top (aqueous phase, about 60% volume).
8. Transfer the RNA aqueous phase (top) to a fresh tube.
9. Save organic phase for DNA and protein isolation.
10. Pricipitate RNA from aqueous phase with isopropanol (isopropyl alcohol, C_3H_8O).
11. Add 0.5 ml isopropanol/ml TRIzol.
12. Incubate 10 min at room temperature.
13. Centrifuge 10 minutes at 4^{o}C by less than 12000 g (10000 rom, r=65 mm).
14. RNA is in bottom as gel-like pellet.
15. Remove and dispose supernatant.
16. Wash RNA pellet once with 75% ethanol: Add 75% ethanol 1 ml/ml TRIzol.
17. Vortex and centrifuge 5 min at 4^{o}C by less that 7500 g (8000 rpm, r=65 mm).
18. Save RNA pellet (air-dry 5-10 min).
19. Dissolve RNA in 0.03–0.06 ml RNase-free water or 0.5% SDS solution.
20. Mix with pipett.
21. Incubate 10 min at 55-60^{o}C.
22. Redissolved in 100% formamide (deionized) and stored at -70^{o}C.
23. De-frozen and for PCR usage.

Precautions for Preventing RNase Contamination:

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.

1. Always wear disposable gloves. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination.
2. Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettes) can be rich sources of RNases.
3. In the presence of TRIZOL® Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that nondisposable glassware or plasticware be RNase-free. Glass items can be baked at 150°C for 4 hours, and plastic items can be soaked for 10 minutes in 0.5 M NaOH, rinsed thoroughly with water, and autoclaved.

Other Precautions:

1. Use of disposable tubes made of clear polypropylene is recommended when working with less than 2-ml volumes of TRIZOL® Reagent.

2. For larger volumes, use glass (Corex) or polypropylene tubes, and test to be sure that the tubes can withstand 12,000 × *g* with TRIZOL® Reagent and chloroform. Do not use tubes that leak or crack.
3. Carefully equilibrate the weights of the tubes prior to centrifugation.
4. Glass tubes must be sealed with parafilm topped with a layer of foil, and polypropylene tubes must be capped before centrifugation.

INSTRUCTIONS FOR RNA ISOLATION:

When working with TRIZOL® Reagent use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor. *Unless otherwise stated, the procedure is carried out at 15 to 30°C, and reagents are at 15 to 30°C.*

Reagents required, but not supplied:

1. Chloroform
2. Isopropyl alcohol
3. 75% Ethanol (in DEPC-treated water)
4. RNase-free water or 0.5% SDS solution [To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water.]

Practical protocol for the RNA isolation using TRIzol

1. HOMOGENIZATION

a. ***Tissues***

Homogenize tissue samples in 1 ml of TRIZOL® Reagent per 50-100 mg of tissue using a glass-Teflon® or power homogenizer (Polytron, or Tekmar's TISSUMIZER® or equivalent). The sample volume should not exceed 10% of the volume of TRIZOL®.

b. ***Cells Grown in Monolayer***

Lyse cells directly in a culture dish by adding 1 ml of TRIZOL® Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRIZOL® Reagent added is based on the area of the culture dish (1 ml per 10 cm^2) and not on the number of cells present. An insufficient amount of TRIZOL®. Reagent may result in contamination of the isolated RNA with DNA.

c. ***Cells Grown in Suspension***

Pellet cells by centrifugation. Lyse cells in TRIZOL® Reagent by repetitive pipetting. Use 1 ml of the reagent per 5-10 × 10^6 of animal, plant or yeast cells, or per 1 × 10^7 bacterial cells. Washing cells before addition of TRIZOL® Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

OPTIONAL: An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 × *g* for 10 minutes at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

2. PHASE SEPARATION

Incubate the homogenized samples for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of TRIZOL® Reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 × *g* for 15 minutes at 2 to 8°C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIZOL® Reagent used for homogenization.

3. RNA PRECIPITATION

Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL® Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no more than 12,000 × *g* for 10 minutes at 2 to 8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. RNA WASH

Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL® Reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500 × *g* for 5 minutes at 2 to 8°C.

5. REDISSOLVING THE RNA

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redissolved in 100% formamide (deionized) and stored at -70°C (5).

RNA Isolation Notes:

1. Isolation of RNA from small quantities of tissue (1 to 10 mg) or Cell (102 to 104) Samples: Add 800 µl of TRIZOL® to the tissue or cells. Following sample lysis, add chloroform and proceed with the phase separation as described in step 2. Prior to precipitating the RNA with isopropyl alcohol, add 5-10 µg RNase-free glycogen (Cat. No 10814) as carrier to the aqueous phase. To reduce viscosity, shear the genomic DNA with 2 passes through a 26 gauge needle prior to chloroform addition. The glycogen remains in the aqueous phase and is co-precipitated with the RNA. It does not inhibit first-strand synthesis at concentrations up to 4 mg/ml and does not inhibit PCR.

2. After homogenization and before addition of chloroform, samples can be stored at -60 to -70°C for at least one month. The RNA precipitate (step 4, RNA WASH) can be stored in 75% ethanol at 2 to 8°C for at least one week, or at least one year at –5 to -20°C.

3. Table-top centrifuges that can attain a maximum of 2,600 × *g* are suitable for use in these protocols if the centrifugation time is increased to 30-60 minutes in steps 2 and 3.

INSTRUCTIONS FOR DNA ISOLATION:

After complete removal of the aqueous phase, as described in the RNA isolation protocol, the DNA in the interphase and phenol phase from the initial homogenate may be isolated. Following precipitation and a series of washes, the DNA is solubilized in 8 mM NaOH. Full recovery of DNA from tissues and culture cells permits the use of TRIZOL® Reagent for the determination of the DNA content in analyzed samples. Simultaneous extraction of genomic DNA allows for normalization of the results of Northern analysis per genomic DNA instead of the more variable total RNA or tissue weight. (Depending on the source, the DNA pellet obtained may require additional purification (e.g., phenol extraction) prior to other applications.

Reagents required, but not supplied:

1. Ethanol
2. 0.1 M Sodium citrate in 10% ethanol
3. 75% Ethanol
4. 8 mM NaOH

Unless otherwise stated, the procedure is carried out at 15 to 30°C.

1. DNA PRECIPITATION

Remove the remaining aqueous phase overlying the interphase, and precipitate the DNA from the interphase and organic phase with ethanol. Add 0.3 ml of 100% ethanol per 1 ml of TRIZOL® Reagent used for the initial homogenization, and mix samples by inversion. Next, store the samples at 15 to 30°C for 2-3 minutes and sediment DNA by centrifugation at no more than 2,000 × *g* for 5 minutes at 2 to 8°C.

Careful removal of the aqueous phase is critical for the quality of the isolated DNA.

2. DNA WASH

Remove the phenol-ethanol supernatant, and if desired, save it for protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M sodium citrate in 10% ethanol. Use 1 ml of the solution per 1 ml of TRIZOL® Reagent used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × *g* for 5 minutes at 2 to 8°C. Following these two washes, suspend the DNA pellet in 75% ethanol (1.5-2 ml of 75% ethanol per 1 ml TRIZOL® Reagent), store for 10-20 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × *g* for 5 minutes at 2 to 8°C.

An additional wash in 0.1 M sodium citrate-10% ethanol solution is required for large pellets containing > 200 μg DNA or large amounts of a non-DNA material.

3. REDISSOLVING THE DNA

Air dry the DNA 5 to 15 minutes in an open tube. (DO NOT DRY UNDER CENTRIFUGATION; it will be more difficult to dissolve.) Dissolve DNA in 8 mM NaOH such that the concentration of DNA is 0.2 – 0.3 μg/μl. Typically add 300 – 600 μl of 8 mM NaOH to DNA isolated from 107 cells or 50 – 70 mg of tissue. Resuspending in weak base is HIGHLY recommended since isolated DNA does not resuspend well in water or in Tris buffers. The pH of the 8 mM NaOH is only ~9 and should be easily adjusted with TE or HEPES once the DNA is in solution. At this stage, the DNA preparations (especially from tissues) may contain insoluble gel-like material (fragments of membranes, etc.) Remove the insoluble material by centrifugation at >12,000 × *g* for 10 minutes. Transfer the supernatant containing the DNA to a new tube. DNA solubilized in 8 mM NaOH can be stored overnight at 4°C; for prolonged storage, samples should be adjusted with HEPES to pH 7-8 (see table) and supplemented with 1 mM EDTA. Once the pH is adjusted, DNA can be stored at 4°C or –20°C.

Quantitation and Expected Yields of DNA

Take an aliquot of the DNA preparation solubilized in 8 mM NaOH, mix it with water and measure the A260 of the resulting solution. Calculate the DNA content using the A260 value for double-stranded DNA. One A260 unit equals 50 μg of double-stranded DNA/ml. For calculation of cell number in analyzed samples, assume that the amount of DNA per 1 × 106 diploid cells of human, rat, and mouse origin equals: 7.1 μg, 6.5 μg, and 5.8 μg, respectively (3).

Applications:

Amplification of DNA by PCR:

After redissolving the DNA in 8 mM NaOH, adjust the pH to 8.4 with 0.1 M HEPES (see table). Add 0.1 to 1.0 μg of the DNA sample to your PCR reaction mixture and perform the standard PCR protocol.

Restriction endonuclease reactions:

Adjust the pH of the DNA solution to a required value using HEPES (see table). Alternatively, samples may be dialyzed against 1 mM EDTA, pH 7 to pH 8.0. Use 3-5 units of enzyme per microgram of DNA. Use the conditions recommended by the manufacturer for the particular enzyme, and allow the reaction to proceed for 3 to 24 h. In a typical assay, 80-90% of the DNA is digestible.

pH Adjustment of DNA Samples Dissolved in 8 mM NaOH:

For 1 ml of 8 mM NaOH use the following amounts of 0.1 M or 1 M HEPES, free acid. Final pH 0.1 M HEPES (μl) Final pH 1 M HEPES (μl).

DNA Isolation Notes:

1. The phenol phase and interphase can be stored at 2 to 8°C overnight.
2. Samples suspended in 75% ethanol can be stored at 2 to 8°C for months.
3. Samples dissolved in 8 mM NaOH can be stored overnight at 2 to 8°C. For long-term storage, adjust the pH to 7-8, and adjust the EDTA concentration to 1 mM.

INSTRUCTIONS FOR PROTEIN ISOLATION:

Proteins are isolated from the phenol-ethanol supernatant obtained after precipitation of DNA with ethanol (step 1, DNA PRECIPITATION). The resulting preparation can be analyzed for the presence of specific proteins by Western blotting (2).

Reagents required, but not supplied:

1. Isopropyl alcohol
2. 0.3 M Guanidine hydrochloride in 95% ethanol
3. Ethanol
4. 1% SDS

1. PROTEIN PRECIPITATION

Precipitate proteins from the phenol-ethanol supernatant (approximate volume 0.8 ml per 1 ml TRIZOL® Reagent) with isopropyl alcohol. Add 1.5 ml of isopropanol per 1 ml TRIZOL® Reagent used for the initial homogenization. Store samples for 10 minutes at 15 to 30°C, and sediment the protein precipitate at 12,000 × *g* for 10 minutes at 2 to 8°C.

2. PROTEIN WASH

Remove the supernatant and wash the protein pellet 3 times in a solution containing 0.3 M guanidine hydrochloride in 95% ethanol. Add 2 ml of wash solution per 1 ml of TRIZOL® Reagent used for the initial homogenization. During each wash cycle, store the protein pellet in the wash solution for 20 minutes at 15 to 30°C and centrifuge at 7,500 × *g* for 5 minutes at 2 to 8°C. After the final wash, vortex the protein pellet in 2 ml of ethanol. Store the protein pellet in ethanol for 20 minutes at 15 to 30°C and centrifuge at 7,500 × *g* for 5 minutes at 2 to 8°C.

3. REDISSOLVING THE PROTEIN PELLET

Vacuum dry the protein pellet for 5-10 minutes. Dissolve it in 1% SDS by pipetting. Complete dissolution of the protein pellet may require incubating the sample at 50°C. Sediment any insoluble material by centrifugation at 10,000 × *g* for 10 minutes at 2 to 8°C, and transfer the supernatant to a fresh tube. The sample is ready for use in Western blotting or may be stored at -5 to -20°C for future use.

Protein Isolation Notes:

1. The protein pellet suspended in 0.3 M guanidine hydrochloride-95% ethanol or in ethanol can be stored for at least one month at 2 to 8°C, or for at least one year at -5 to -20°C.
2. The following protocol is an alternative approach that allows for more efficient recovery of proteins. Dialyze the phenol-ethanol supernatant against three changes of 0.1% SDS at 2 to 8°C. Centrifuge the dialyzed material at 10,000 × *g* for 10 minutes. Use the clear supernatant for Western blotting.
3. Proteins may be quantified by the Bradford method as long as the concentration of SDS is low enough (<0.1%) so that it will not interfere. Methods that do not have detergentinterface problems, and that do not rely on A260/A280 measurements may be used (traces of phenol may cause overestimation of protein concentrations).

For RNA ISOLATION, Expected yields of RNA per mg of tissue or 1 × 106 cultured cells:

1. Liver and spleen, 6-10 µg
2. Kidney, 3-4 µg
3. Skeletal muscles and brain, 1-1.5 µg
4. Placenta, 1-4 µg
5. Epithelial cells (1 × 106 cultured cells), 8-15 µg
6. Fibroblasts, (1 × 106 cultured cells) 5-7 µg

For DNA ISOLATION, Expected yields of DNA per mg of tissue or 1 × 106 cultured cells:

1. Liver and kidney, 3-4 µg
2. Skeletal muscles, brain, and placenta 2-3 µg
3. Cultured human, rat, and mouse cells (1 × 106), 5-7 µg
4. Fibroblasts, 5-7 µg

Figure 1 gives a brief steps for the TRIzol usage on RNA isolation, as an example of RNA isolation from human platelet with the scheme description, for readers to sue it as a reference.

An example of RNA isolation from human platelet:

Platelet pellet

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Trizol 5-10X10^6 cells/1 ml TRIzol

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5 minutes incubate at room temperature

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0.2 ml chloroform/1 ml TRIzol

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Shake tubes vigorously by hand for 15 seconds

↓

Incubate at room tempearture for 3 minutes

↓

Centrifuge 15 minutes at 4^{o}C at less than 12,000 g (10000 rpm, r=65 mm)

↓

RNA is in top (aqueous phase, about 60% volume)

↓

Transfer the aqueous phase to a fresh tube

↓

Save organic phase for DNA and protein isolation

↓

Pricipitate RNA from aqueous phase with isopropanol (isopropyl alcohol, C_3H_8O)

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Add 0.5 ml isopropanol/ml TRIzol

↓

Incubate 10 minutes at room temperature

↓

Centrifuge 10 minutes at 4°C by less than 12000 g (10000 rom, r=65 mm)

↓

RNA is in bottom as gel-like pellet

↓

Remove and dispose supernatant

↓

Wash RNA pellet once with 75% ethanol

↓

Add 75% ethanol 1 ml/ml TRIzol

↓

Vortex and centrifuge 5 minutes at 4°C by less that 7500 g (8000 rpm, r=65 mm)

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Save RNA pellet (air-dry 5- 10 minutes)

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Dissolve RNA in 0.03–0.06 ml RNase-free water or 0.5% SDS solution

↓

Mix with pipett

↓

Incubate 10 minutes at 55-60°C

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Redissolved in 100% formamide (deionized) and stored at -70°C

De-frozen and for PCR usage

Figure 1. TRIzol steps for RNA isolation

To get better treatment of human blood, the following is platelet isolation by Ficoll-Pague method:

1. Human bloods were collected in 2.7-ml tubes containing 2.7 ml of 3.2% (0.109 M) Na citrate.
2. About 2.5 ml of blood was collected in each tube and 8 tubes for each drawing, and the total blood for each draw was about 20 ml.
3. Each blood drawing was done by pre-dialysis and post-dialysis separately (about 6 hours for the dialysis), and each patient was drawn blood four times totally (one pre-dialysis and one post-dialysis for first drwaing, and another one pre-dialysis and one post-dialysis for second drawing).
4. Centrifuge at 800 rpm for 15 min, at room temperature.
5. Collect platelet-rich-plasma (PRP) (supernatant).
6. Put PRP into the 14-ml Corning centrifuge tube that has 3 ml of Ficoll-Pague Premium solution (slowly along side).
7. Centrifuge at 1350 rpm for 35 min, at room temperature.
8. Collect PRP in the top of the tube.
9. Centrifuge at 4000 rpm for 30 min, at room temperature.
10. Collect platelet (pellet) and freeze the supernatant for protein measurement.
11. Wash platelet with 5 ml of physiological buffered solution (PBS).
12. Centrifuge at 4000 rpm for 30 min, at room temperature.
13. Keep platelet (pellet) at freezer (-70°C) until RNA isolation.

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