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RNJia Phenol-free PB Kit (With RBC Lysis Buffer)
RNJia Phenol-free PB Kit (No RBC Lysis Buffer)

RNA and total RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

PBMC (Peripheral Blood Mononuclear Cell)

Whole Blood

WBC (White Blood Cell)

By ROJE

Edition, 01/2022

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

Contents

Kit Content

Component	100 preps
RLB	2 x 35 ml
TWB1 (concentrate)	2 x 16 ml
TWB2 (concentrate)	2 x 15 ml
Nuclease-free RBC Lysis buffer Just included in RNJia Phenol-free PB Kit, (With RBC Lysis Buffer)	2×375 ml
Nuclease-free Water	10 ml
Micro Column	100
Collection Tube	300

Storage

Shipment condition is checked by ROJETechnologies. After arrival, RNSol H Reagent should be kept dry, at 2-8 °C and all remaining reagent should be kept at room temperature (15-25 °C). When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

RNJia Phenol-free PB Kit provides the components and procedures necessary for purifying RNA and miRNA from PBMC, whole blood and WBC. Notice that, RNJia Phenol-free PB Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study RNA experiments guideline, before starting their work.

Guarantee & Warranty

ROJETechnologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our technical support team. If problem is due to manufacturing process, ROJE team will replace the Kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell this kit or any components. For information about out licensing or distributors contact ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com

Quality Control

RNJia Phenol-free PB Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at www.rojetechnoloes.com

Description

RNJia phenol-free PB Kit provides a time-saving, reliable and meticulous method for RNA isolation (including miRNA) from peripheral blood mononuclear cell, WBC and whole blood. RNJia phenol-free PB Kit is based on Micro column technology for isolation of concentrated, highly purified and intact RNA, which is suitable to be used for a variety of downstream applications such as Northern blot experiments, Real-time PCR, RNA sequencing, Microarray etc.

Procedure

The RNJia phenol-free PB Kit is designed for isolating all forms of RNA molecules from up to 1×10^7 WBCs. Fresh or frozen samples should be lysed and homogenized. Then, RNA binding to the silica membrane is achieved selectively, by addition of ethanol to the lysate. Contaminants are removed by two specific kind of washing buffers. Pure RNA is finally eluted in nuclease-free water. Isolated RNA is ready to use in downstream applications. It has A260/A280 ratios of 1.8–2.3 by spectrophotometer, confirming high purity.

Equipment & Reagents to Be Supplied by User

- Absolute ethanol
- 1.5ml RNase free microcentrifuge tubes
- 2-Mercaptoethanol
- RNase free pipets and pipet tips
- Vortex
- Thermoblock or water bath

- TissueLyser/ Mortar and pestle/ Homogenizer
- Syringe and needles

Applications

The isolated RNA and miRNA can be used in many downstream applications:

- RNA Sequencing
- cDNA synthesis
- Poly A+ RNA selection
- Microarrays
- RT-PCR and real-time RT-PCR
- Northern, dot blot analysis

Features

Specific features of RNJia Phenol-free PB Kit are listed here in Table 1.

Table 1: RNJia Phenol-free PB Kit features and specifications

Features	Specifications
Elution volume (µl)	30 µl
Technology	Silica technology
Main sample type	PBMC / Whole blood /WBC
Processing	Manual
Purification of total RNA, miRNA, Poly A+ mi-RNA, DNA or protein	Yes
Sample Amount	Up to 1.5 ml whole blood (1 X 10^7 cells)
Operation time per reaction (min)	45 Min
Typical yield (µg)	4.5-20µg
Average purity	A260/A280= 1.8-2.3
Size of purified RNA	RNA ≥ 200 bp
Enzyme	No

Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended starting material to use with determined RLB volumes is written in Table 2.

Table 2: Appropriate sample size and amount of RLB

Whole blood (ml)	Number of WBCs	Amount of RLB (µl)
Up to 0.5	Up to 2 x 10^6	400
0.5 to 1.5	2 x 10^6 to 1 x 10^7	700

Sample Storage and Preparation

Preparation: Sample preparation is composed of 2 steps, disruption and homogenization. First step is disruption of cell walls, plasma membranes of cells and organelles. Inefficient disruption results in RNA yield reduction. This can be done with one or cooperation of two methods depending on the tissue type. These methods include RNSol H Reagent, Mortar and Pestle, Tissuelyser etc. Viscosity reduction can be accomplished by Homogenization. The aim is to create a homogeneous lysate. Sometimes these 2 steps happen simultaneously. For more information refer to Table 3.

> **Note:** After disruption and homogenization in RNSol H Reagent (lysis buffer), samples can be stored at –70°C for months.

Table 3. Disruption and homogenization for different sample types

Sample type	Disruption	Homogenization
Animal Cells, (WBC, PBMC…)	• RLB • Vortexing	• Homogenizer • Syringe and needles

Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 5. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 5: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
TWB1	16 ml	24 ml	40 ml
TWB2	15 ml	45 ml	60 ml

Maximize RNA Yield

To obtain higher yield of RNA, it is important to follow protocol carefully and pay attention to sample size table and suitable RLB amount. Notice that all samples must be completely homogenized during cell lysis for maximum yield. It is good to know that:

- Avoid Freezing and thawing samples, which may result in decreasing RNA yield, compared to isolating RNA from fresh samples.

Genomic DNA Contamination

RNJia phenol-free PB Kit is designed to selectively isolate RNA. However, if further DNA removal is intended, it is recommended to use DNase Treatment kits, which are available from different suppliers.

Protocols

Phenol-Chloroform Free Based Protocols

Protocol: Isolation of RNA (PBMC, WBC, and Whole blood)

Sample Type: PBMC (Peripheral Blood Mononuclear Cell), WBC (White Blood Cell), and Whole blood

Some tips to know:

- All steps, before applying the sample to Micro Column, are carried out on the ice.

Before using the protocol for the first time, add 2-Mercaptoethanol to RLB, 1% of RLB volume. It is better to calculate it every time you test and add 1% of 2ME to it for higher efficiency. It is 693 µl of RLB and 7 µl of 2ME for each sample test. 2-Mercaptoethanol is commercially available and due to safety issues not provided in the kit.

Attention: It is recommended to prepare it freshly. It is not stable for more than 2 weeks.

- If RLB forms a precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage conditions and will not influence the efficiency of the buffer
- Do Not forget to add the appropriate amount of ethanol (molecular biology grade, 96–100%) to TWB1 and TWB2 as indicated on the bottle, before using them for the first time. Refer to the washing buffer preparation section.
- If working with RNA for the first time, read Appendix 1 carefully.

Process

1. Collect 0.5 to 1.5 ml of blood into EDTA tubes. Add Five volumes of Nuclease-free RBC Lysis Buffer. Vortex the tube for 15s and incubate at 4 ºc for 20 min or incubate at freezer -20 ºc for 10 min.
2. Pulse vortex and five times invert every 3 min during incubation to intersperse the sample.
3. Collect the WBCs by centrifugation at 400 x g for 10 min at 4ºc.
4. Discard the supernatant; add two volumes of Nuclease-free RBC Lysis Buffer to the pellet, vortex until the pellet is dissolved completely.
5. Collect the WBCs by centrifugation at 400 g for 10 min at 4ºc.

   Note: To remove erythrocyte cells, you can use 1 ml of peripheral blood into the Falcon 15 and add 5 ml of RBC lysate to it, vortex for 15 seconds, place in the freezer for 15 minutes, and 10 times invert the Falcon every 3 minutes. then centrifuge at 1500 rpm for 10 minutes

   After that, discard the supernatant and add 1 ml of RBC to the falcon Pipet it well and then transfers all the contents to a sterile 1.5 vial.

   centrifugation at 1750 rpm for 10 minutes
6. Discard the supernatant. Disrupt the cells by adding RLB (prepared with 2-βME). Loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RLB vortex or pipet to mix. for example, for the volume of up to 500 µl of blood, add the amount of 400 µl of RLB and for the volume of the blood sample up to 1.5 ml/liter, add the amount of 700 µl of RLB.

After adding the appropriate amount of RLB, use a Micro pestle followed by a homogenizer or syringe needle to homogenize the sample for 1min.

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

Note: For previously isolated PBMCs and WBCs, start from step 6.

7. Centrifuge the lysate for 3 min at 15000 rpm. Carefully remove the supernatant by pipetting, and transfer it to a new RNase-free microcentrifuge tube.
8. Add one volume of 70% Cold Ethanol to the homogenized lysate, and mix well with a calm pipette.

   Note: When purifying RNA from isolated cells, precipitates may be visible after the addition of ethanol. This does not affect the procedure.
9. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 15s at 12000 rpm. Discard the flow-through. Reuse the collection tube in step 10
10. add the remainder of the sample to the column and centrifuge for 15s at 12000 rpm Discard the flow-through new collection
11. Add 700 µl TWB1 to the Micro Column. Centrifuge for 15s at 12000 rpm at room temperature. Discard the flow-through.
12. Add 600 µl TWB2 to the Micro Column. Centrifuge for 15s at 12000 rpm at room temperature. Discard the flow-through.
13. Add 500 µl TWB2 to the Micro Column. Centrifuge for 3 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
14. Place the Micro Column in a new 1.5 ml microtube. Add 30µl RNase-free water directly to the Micro Column membrane and incubation in RT for 3-5min then Centrifuge for 75s at 13000 rpm to elute the RNA.

    Note: If the expected RNA yield is more than the yield from the previous step, put the Micro Column on a new microtube and add another 30µl RNase-free water. Centrifuge for 1 min at 13000 rpm. However, it is possible to pass the flow-through from step 13 once more to obtain RNA with a higher concentration.

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
Low yield	Inappropriate sample storage condition	• Avoid freezing and thawing of samples, which results in reduced RNA Yield.
	Incomplete cell lysis	• Too much starting material results in low RNA yield. To optimize the results, refer to Table 2.
	Ethanol from the washing buffer is present in elution	• Preform another centrifugation before rehydration step to ensure no remaining trace of ethanol on column. • Carefully remove the column from the collection tube so that the column does not contact the flow-through.
	RNA elution is incomplete	• Perform rehydration step once more, by adding another 30-100µl rehydration buffer to the column and before centrifugation, incubate 5 min at room temperature. • Check that all previous steps are done appropriately.
Degradation	RNase contamination	• All buffers have been tested and are guaranteed RNase-free. RNases can be introduced during use. Refer to appendix 1 for more information.
Low 260/280 ratio	Insufficient disruption and homogenization	• As a guide for better disruption and homogenization, based on sample type refer to sample preparation guidelines.
	RNA was diluted in low pH water	• Use 10 mM Tris-HCl with pH ≥ 7.5, or nuclease free water with pH ≥ 7.5.
	DNA contamination	• Follow precisely the respective protocol, If RNA purification is still problematic further do DNase treatment.
	Protein contamination	• This is often due to exceeding the amount of starting material. Follow precisely the respective protocol; if RNA purification is still problematic further reduce the amount of starting material. • Remove the aqueous phase precisely.
DNA contamination in	No DNase treatment	• Perform DNase treatment.

downstream application	No incubation with TWB1	• Incubate the Micro column for 5 min at room temperature after addition of TWB1 and before centrifuging
Not performing well in downstream application	Ethanol carryover	• Preform another centrifugation before rehydration step to ensure no remaining of ethanol on column.
	Salt carryover	• Between washing steps, eliminate remaining flow-through from the rim of collection tube, by blotting on clean paper towels.
Clogged Column	Maximum amount of tissue exceeds kit specifications	• Refer to specifications to determine if the amount of starting material falls within kit specifications.
	The sample is too large	• Use fewer starting material. The problem can be solved by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
	Centrifuge at low temperature	• The centrifugation temperature should be 20–25°C. Make sure that the centrifuge temperature is set at 25°C.
	Centrifugation before adding Ethanol is not performed (for animal tissue)	• Centrifuge the lysate before adding ethanol, and use supernatant for next step.

Ordering Information

Category	Product Name	Cat No.	Size
	RNJia Phenol-free PB Kit (No RBC Lysis Buffer)	RN983052	100 preps
	RNJia phenol-free PB Kit (With Nuclease-free RBC Lysis Buffer)	RN983057	100 preps
	RNJia Kit	RN983006	100 preps
	RNJia Fibrous Kit	RN983025	50 preps
	RNJia Bacteria Kit	RN983022	50 preps
	RNJia Bacteria Kit	RN983023	100 preps
	MiRJia Lipid Kit	RN003078	100 preps
	MiRJia Kit	RN003079	100 preps
	RNaseLag	RN983007	100ml
	RNZO	RN983048	25ml
	RNA Loading Set	LD983007	100 Preps
	RNSol H Reagent	RN983061	100ml

Technical Assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team is composed of highly trained, experienced scientists; who can troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select a suitable product.

- Contact our technical support at any time by selecting one of these ways:
- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website (www.ROJETechnologies. com)

Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Appendix 1: Handling RNA

RNA is highly sensitive to degradation due to the ubiquitous presence of RNases in the environment. unlike DNases, which require metal ions for activity, RNases do not require cofactors for their enzymatic activity and can maintain activity even after prolonged boiling or autoclaving. Since RNases are active enzymes, and are too hard to be deactivated, do not use

any plasticware or glassware without first removing possible RNase pollution. Special precautions should be taken when working with RNA. All reagents and equipment must be specially treated to inactivate RNases prior to use.

The following are some tips and techniques to remember when working with RNA:

General Tips

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory mobilizations. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
- Treat surfaces of benches with commercially available RNase inactivating agents such as RNZO (Order by cat No RN983018 and RN983019).
- Treat non-disposable glassware and plasticware before use, to ensure that it is RNase-free. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA, and RNase-free water.
- Use disposable RNase-free plasticware to decrease the possibility of sample contamination.
- Keep purified RNA on ice when aliquots are pipetted for downstream applications.
- Glassware should be immersed in freshly prepared %0.1 (v/v) DEPC in water or ethanol for several hours, followed by draining and autoclaving.

Appendix 2: RNA Storage Condition

RNA samples are commonly stored at −20 °C or −80 °C, eluted in RNase-free water. Under these conditions, RNA is not degraded after 1 year.

RNA Quality

The ratio of absorbance at 260nm and 280nm is used to assess the purity of RNA using UV absorption, by a spectrophotometer or Nanodrop. For pure RNA, A260/A280 ratios should be around 2.1. If the ratio is noticeably lower in either case, it may indicate the presence of protein or other contaminants that absorb strongly at or near 280nm. High A260/A280 purity ratios are not indicative of an issue.

Appendix 3: RNA Integrity

The integrity of RNA molecules is important for experiments that try to reflect the snapshot of gene expression of RNA isolation. The least expensive method for checking RNA integrity is to run the RNA on a %1 DGE agarose gel and examine the ribosomal RNA (rRNA) bands. The upper ribosomal band (28S in eukaryotic cells and 23S in bacterial cells) should be about

twice the intensity of the lower band (18S in eukaryotic cells and 16S in bacterial cells) and should be sharp and tight.

Denaturing agarose gel electrophoresis

Formaldehyde is used to keep the RNA from denaturation, and when RNA is heated in the presence of formaldehyde all secondary structures are eliminated. Therefore, gel electrophoresis of RNA in agarose gels containing formaldehyde provides a good denaturing gel system. Formaldehyde is a potential carcinogen. Take appropriate safety measures and wear gloves when handling.

Gel preparation

- Prepare %1.2-2 gel with TAE 1x buffer (%5 formaldehyde).
- Pour the gel using a comb that will form wells large enough to accommodate at least 20µl.
- Assemble the gel in the tank.
- Use TAE 1x buffer (%5 formaldehyde) as the tank buffer.

RNA sample preparation

- Add 10µg of purified RNA to 6µl of ROJE sample buffer (in RNA Loading Set, order by cat No LD983007) and incubate at 70 °C for 3 min.
- Add 3µl Hinari to the mixture of RNA and sample buffer and mix well by pipetting.
- Then run the mixture in the prepared gel.

Electrophoresis

Load the gel and electrophorese at 5-6 V/cm until the loading dye at least has migrated 2/3 the length of gel.

Results

Visualize the gel on a UV transilluminator. The 28S and 18S ribosomal bands should be sharp and intense. The intensity of the 28S band should be twice more than the 18S band. The following Figure shows different RNA samples, isolated by RNJia kit and run by DGE.

Note: If DNA contamination is present, the high molecular weight smear will be appeared above the 28S band.

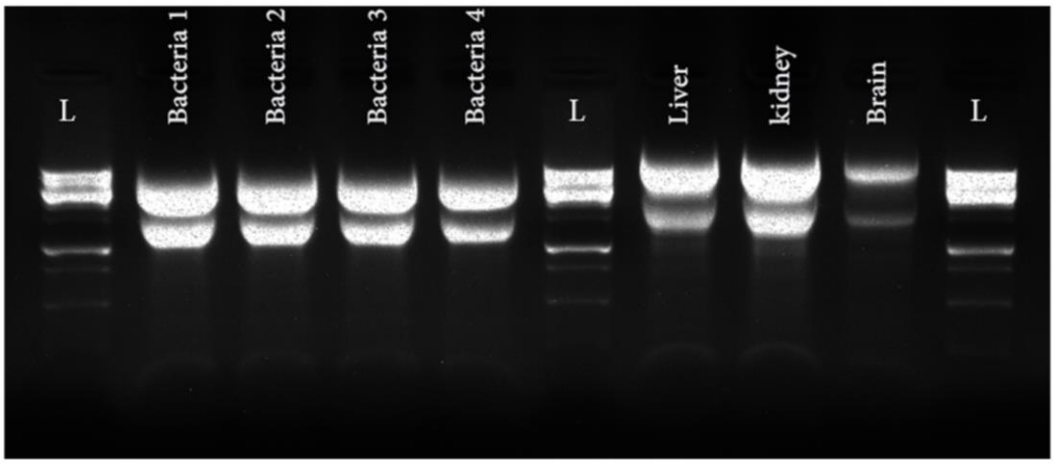

Figure 1. Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10 μg RNA was pretreated by ROJE Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

Appendix 4: DNA Contamination

Appendix 5: Convert RPM to RCF (centrifuge)

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})(r)}}$$

Where **RCF** = required gravitational acceleration (relative centrifugal force in units of g); **r** = radius of the rotor in cm; and **RPM** = the number of revolutions per minute required to achieve the necessary g-force.

Appendix 6: Cell Count by a Hemocytometer

Sample preparation

First, resuspend the cell in flask, and then quickly remove your aliquot of cells. For a cell culture growing in a Petri dish, you need to resuspend the cells growing on the bottom of the dish by gently using a pipette to remove cells and media from a dish and then gently expelling them back into the dish. This aspiration of the contents of the Petri dish should be completed several times, each time expelling the cells and media while moving the pipette across the bottom of the dish to gently discharge cells growing on the entire surface. After resuspension, the aliquot is quickly removed from the vessel before the cells settle to the bottom again. For cell culture applications cells are often stained with Trypan Blue so that dead cells can be distinguished from live cells. For example, a 0.5ml suspension of cells would be removed from the Petri dish and mixed with 0.5ml Trypan Blue solution in an Eppendorf or small test tube. Trypan Blue is a stain that selectively stains dead cells.

Loading Sample

Load your sample quickly and smoothly. It is important to handle the hemocytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide. Always clean the slide before you load the sample by rinsing the slide and cover slip with %70-95 ethanol. Air-dry or gently wipe the slide and cover slip with lens paper. Place the clean and dry slide on your work surface and place the coverslip on top to cover the reflective surfaces. If you have diluted your cells in a test tube, invert the tube several times to resuspend the cells. Using a micropipette, quickly and smoothly without interruption, add 10µl of your cell suspension (or 1 drop from a transfer pipette) to the V-shaped groove on each side of the hemocytometer. If your sample moves into the gutters, you may not have loaded the sample in the correct location or you may have used too large of an aliquot.

Estimating cell density

count all of cells within each of the four large quadrants in the four corners of each counting chamber on the hemocytometer (see Figure 2). Count all of the cells within each quadrant except those on the far-right edge and lower bottom edge.

Calculate the cell density by this formula:

Average number of cells × dilution factor× 10^4

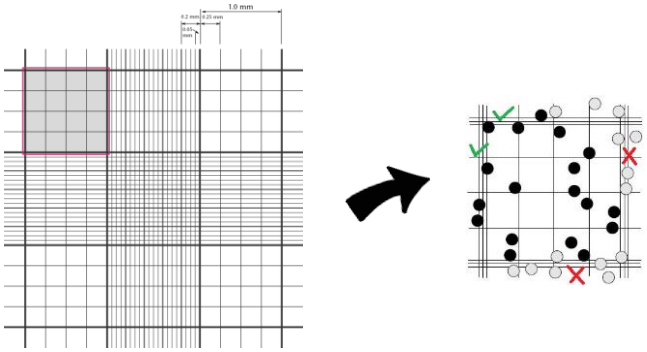

Figure2. Cell counting with hemocytometer

Appendix 7: Preparation of Phosphate Buffered Saline (PBS)

Dissolve the components in about 800mL dH2O. Adjust the pH to 7.4 with HCl. Adjust the volume to one liter. Sterilize by autoclaving. Store at room temperature.

Table 7. PBS preparation

Concentration	Component	G/litter
137mM	NaCl	8g
2.7mM	KCl	0.2g
10mM	Na2HPO4	1.42g
1.8mM	KH2PO4	0.25g

Factory address

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ROJETECHNOLOGIES has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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