VeZol Reagent

R411

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

VeZol Reagent is intended for the isolation of high-quality total RNA from cultured cells, animal tissues, and plant tissues. VeZol Reagent is a monophasic solution of phenol and guanidine isothiocyanate supplemented with optimized proprietary components for more effective sample disruption and RNase inhibition. After rapid sample lysis with VeZol Reagent and chloroform extraction, the lysate separates into an upper aqueous phase (containing RNA), an interphase, and a lower red organic phase, and total RNA can be obtained in high yield and purity by isopropanol precipitation and ethanol wash. The resulting total RNA can be used directly for RT-PCR, RT-qPCR, Northern Blot, Dot Blot, *in vitro* translation, high-throughput sequencing, and other molecular biology experiments.

02/Components

Components	R411-01	R411-02
VeZol Reagent	100 ml	200 ml

03/Storage

Store at 2 ~ 8°C and protect from light. Transport at room temperature.

04/Applications

It is applicable for a variety of cultured cells, animal tissues, and plant tissues.

05/Self-prepared Materials

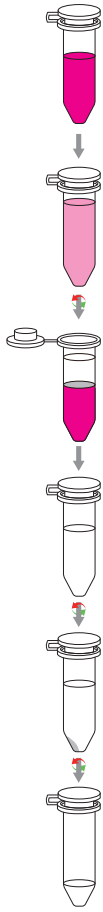
Chloroform, isopropanol, 75% ethanol (prepared with RNase-free ddH_2O), RNase-free ddH_2O, RNase-free centrifuge tubes, and RNase-free glycogen (optional).

06/Notes

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

1. This product contains phenol, which is toxic and corrosive. Wear protective equipment, such as protective clothing, gloves, goggles, and face shields, while handling the reagent. In case of eye contact, flush immediately with large amounts of water and seek medical attention. In case of skin contact, flush immediately with large amounts of detergent and water. If discomfort persists, seek medical attention.
2. The key to RNA extraction is preventing RNase contamination. RNase, which is prevalent in the environment and extremely stable, can rapidly degrade RNA, even in trace amounts. Therefore, take all necessary precautions as per the conventional RNA extraction procedure, including wearing a mask and sterile disposable gloves, working in a separate clean area, and using RNase-free labware.

07/Mechanism & Workflow



Sample lysis: Add 1 ml of VeZol Reagent, vortex, and incubate at room temperature for 5 min

Chloroform extraction: Add 1/5 volume of chloroform, shake vigorously, and incubate at room temperature for 5 min

Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 15 min

Supernatant transfer: Pipette 500 µl of the upper aqueous phase

RNA precipitation: Add an equal volume of isopropanol, mix by inversion, and incubate at room temperature for 10 min

Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 10 min and discard the supernatant

Ethanol wash: Add 1 ml of 75% ethanol

Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 5 min and discard the supernatant

RNA dissolution: Air dry and add 20 - 100 µl of RNase-free ddH_2O to dissolve the precipitation

Fig 1. Workflow of VeZol Reagent

08/Experiment Process

08-1/Sample Processing

The following table lists the maximum amounts of samples that can be fully lysed with 1 ml of VeZol Reagent:

Adherent cells	1×10^5 - 1×10^7 cells (3.5 cm diameter dish, about 10 cm^2 culture area)
Suspension cells	5×10^6 - 1×10^7 cells
Animal tissue	50 - 100 mg
Plant tissue	50 - 100 mg

▲ Too much sample may lead to insufficient lysis and decrease product purity.

▲ The sample volume should not exceed 10% of the volume of VeZol Reagent.

Adherent cells

1. Discard the culture medium and rinse once with 1 × PBS.
2. Add 1 ml of VeZol Reagent per well of a regular 6-well cell culture plate or per 3.5 cm diameter dish (approximately 10 cm^2 culture area). Allow the VeZol Reagent to fully cover the cell layer, and then detach the cells by pipetting repeatedly.
   ▲ Firmly adherent cells (cell clumps) can be detached with a cell scraper or a clean pipette tip. Alternatively, treat the cells with trypsin before adding VeZol Reagent, and then follow the steps for suspension cells.
3. Transfer the mixture to a 1.5 ml centrifuge tube, pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.

Suspension cells

1. Centrifuge at 2,300 rpm (500 × g) and 4°C for 2 - 5 min and discard the supernatant to collect the cells.
2. Add 1 ml of VeZol Reagent per 5 × 10^6 - 1 × 10^7 cells.
3. Vortex or pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.
   ▲ Frozen cells should be vortexed immediately after the addition of VeZol Reagent to avoid incomplete lysis.

Animal/plant tissues

◇Liquid nitrogen grinding

1. Retrieve cryopreserved tissue or flash freeze fresh tissue in liquid nitrogen. Quickly transfer the sample to a mortar precooled with liquid nitrogen. Grind with a pestle while adding liquid nitrogen until the sample is ground into powder (with no obvious granules).
   ▲ Insufficient grinding may impair RNA yield and quality.
   ▲ Liquid nitrogen grinding is recommended for plant samples.
2. Transfer the powdered sample into a centrifuge tube, add 1 ml of VeZol Reagent per 50 - 100 mg of tissue, and vortex until there are no obvious clumps. Incubate at room temperature for 5 min.
3. (Optional) For lipid-rich samples, centrifuge at 11,200 rpm (12,000 × g) and 4°C for 5 min, remove the top layer of fat, and transfer the clear supernatant to a new RNase-free centrifuge tube.

◇Homogenization

1. Add 1 ml of VeZol Reagent per 50 - 100 mg of fresh tissue, and homogenize with a glass or electric homogenizer until there are no obvious tissue blocks. Incubate at room temperature for 5 min.
2. (Optional) For lipid-rich samples, centrifuge at 11,200 rpm (12,000 × g) and 4°C for 5 min, remove the top layer of fat, and transfer the clear supernatant to a new RNase-free centrifuge tube.

08-2/Total RNA Extraction

1. Add 1/5 volume of chloroform to the above lysate. Shake vigorously for 15 sec to obtain an emulsion, and incubate at room temperature for 5 min.
   ▲ For genomic DNA-rich samples, add 5 μl of glacial acetic acid per 1 ml of VeZol Reagent after sample lysis and mix well before adding chloroform.
   ▲ Ensure that a homogeneous emulsion is formed so that proper phase separation can be achieved after centrifugation.
2. Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 15 min.
   ▲ Centrifuge at a low temperature to avoid increased genomic DNA contamination.
3. Carefully take out the centrifuge tube. The mixture separates into three layers: an upper aqueous phase (containing RNA), an interphase, and a lower red organic phase. Carefully transfer the upper aqueous phase (about 500 μl) to a new RNase-free centrifuge tube. Do not disturb the interphase.
   ▲ The volume of the upper aqueous phase is about 60% of the volume of the VeZol Reagent used. If 1 ml of VeZol Reagent is used for RNA extraction, the upper aqueous phase is approximately 600 μl.
4. (Optional) For small amounts of starting sample (<10^6 cells or <10 mg of tissue), add 5 - 10 μg of RNase-free glycogen (not provided) to the upper aqueous phase.
5. Add an equal volume of isopropanol, mix by inversion, and incubate at room temperature for 10 min.
6. Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 10 min, and a white precipitation will usually be observed. Carefully discard the supernatant.
   ▲ The precipitation will be barely visible for samples with a low RNA content. Proceed with the workflow.
   ▲ To minimize residual impurities, discard supernatant as completely as possible in this step. After removing the supernatant, the RNase-free tube can be left inverted on a clean blotting paper for 1 min. Do not discard or pipette out the RNA precipitation.
7. Add 1 ml of 75% ethanol (prepared with RNase-free ddH_2O). Gently flick the tube to loosen the precipitation, and invert the tube a few times.
8. Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 5 min and carefully discard the supernatant.
   ▲ To minimize residual impurities, discard supernatant as completely as possible in this step. Take care not to lose the precipitation. It is recommended to discard most of the supernatant, spin the tube briefly to collect all the liquid to the bottom, and then remove the remaining liquid with a pipette. Take care not to lose or disturb the RNA precipitation.
9. Air dry the precipitation for 2 - 5 min at room temperature in a clean environment. Add 20 - 100 μl of RNase-free ddH_2O to dissolve the precipitation and vortex at room temperature or pipette up and down for thorough dissolution. The extracted RNA can be aliquoted and stored at -85 ~ -65°C for long-term storage or -30 ~ -15°C for short-term storage.
   ▲ Do not overdry the precipitation, or the RNA will be hard to dissolve.
   ▲ RNA dissolution can be facilitated by incubating in a 55 ~ 60°C water bath for 10 - 15 min.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Low RNA yield	1.Incomplete sample lysis or homogenization	Reduce the sample input.
		Cut the tissue sample into small blocks and grind or homogenize thoroughly to ensure complete lysis.
	2.Incomplete dissolution of RNA precipitation	Incubate in a 55 ~ 60°C water bath for 10 - 15 min to aid RNA dissolution.
RNA degradation	1.RNase contamination	Ensure that all extraction reagents and labware are free from RNase contamination. Thermal-resistant labware can be baked at 150°C for 4 - 6 h to remove RNase, and other equipment can be soaked in 0.1% DEPC-treated ddH_2O overnight.
		Take appropriate preventive measures, including wearing masks and sterile disposable gloves and working in a separate clean area.
	2.Improper sample storage or treatment	If RNA is not extracted right away, dissected tissues should be immediately flash-frozen in liquid nitrogen and either stored there or at -85 ~ -65°C. Do not freeze the tissue directly at -85 ~ -65°C, as slow freezing will leave enough time for endogenous RNases to degrade RNA.
		If flash-freezing in liquid nitrogen is not feasible, the fresh tissue can be immersed in RNA Keeper Tissue Stabilizer (Vazyme #R501) and stored at room temperature for one week, 2 ~ 8°C for one month, or -30 ~ -15°C (or -85 ~ -65°C) for a longer time.
		Add VeZol Reagent immediately after sample retrieval and promptly proceed to the subsequent steps to avoid RNA degradation by endogenous RNase.
	3.High endogenous RNase content in sample	Reduce the sample input.
		Use liquid nitrogen grinding to lyse the sample, keeping it at an ultra-low temperature at all times.
		Use precooled VeZol Reagent (2 ~ 8°C) for sample lysis.
	4.Improper RNA product storage	Take a small amount of the product for testing, and store the rest in aliquots at -85 ~ -65°C. Use a higher voltage and shorter run time for electrophoresis (220 V and 10 min recommended), and chill the electrophoresis buffer in an ice bath to prevent RNA degradation during the run.
Genomic DNA contamination	1.Incorrect centrifugation temperature	Centrifuge at low temperatures (2 ~ 8°C) after adding chloroform.
	2.Improper transfer of the aqueous phase	Take care to transfer only the aqueous phase, and some liquid in the aqueous phase can be left. Do not disturb the interphase or lower phase to avoid genomic DNA contamination.
	3.High genomic DNA content in sample	After sample lysis, add 5 µl of glacial acetic acid per 1 ml of VeZol Reagent and mix well before proceeding to the next step.
Low purity	1.Residual impurities and salt ions	Increase the number of washes and precipitations with 75% ethanol (prepared with RNase-free ddH_2O).
		Discard supernatant as completely as possible in the relevant steps (08-2/Total RNA Extraction/Steps 6 and 8).
Precipitation invisible after isopropanol addition and centrifugation	1.Low RNA content or tissue input	Add 5 - 10 µg of RNase-free glycogen to the upper aqueous phase as a co-precipitant with RNA.
		After adding isopropanol (08-2/Total RNA Extraction/Step 5), leave the reaction system at 2 ~ 8°C or -30 ~ -15°C for 10 - 30 min before centrifugation. Take care not to lose or disturb the RNA precipitation when discarding the supernatant (08-2/Total RNA Extraction/Steps 6 and 8).
	2.High metabolite content in sample	Some tissue samples contain high levels of metabolites, causing the RNA to be smeared along the centrifuge tube wall instead of forming a compact precipitation. Slowly collect the supernatant at the liquid surface, taking care not to lose or disturb the RNA.

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