Wizol™ Reagent

RUO For Research Use Only

REF W76100

DESCRIPTION

The Wizol™ Reagent is a complete and ready-to-use reagent for the isolation of total RNA from tissue, cultured animal and bacterial cells, and cell-free fluids such as serum and plasma. The Wizol™ Reagent contains guanidinium, a powerful chaotropic agent effective for rapidly inactivating nucleases, and phenol, an organic solvent used to denature and separate proteins and DNA from RNA.

The Wizol™ Reagent ensures total RNA with high yield and good quality from samples of unlimited size. If a larger sample is required, the buffer volume can be scaled proportionately, making this reagent not only very user friendly but also highly versatile. The total RNA is ready for use in RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

KIT CONTENTS

Contents	W76100
Wizol™ Reagent	100 ml

STORAGE CONDITIONS

- Store at 2~25ºC and protect from the light.
- Wizol™ is stable for 24 months when properly stored.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY USER

- 75% ethanol
- Isopropanol
- RNase-free water
- Chloroform
- 1.5 mL microcentrifuge tubes
- Sterile RNase-free pipette tips and Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

PRECAUTIONS

- Wizol™ Reagent contains phenol (corrosive liquid/poison) and guanidine isothiocyanate (irritant). Causes burns and can be fatal. When working with Wizol™ Reagent, use gloves and eye protection (face shield, safety goggles). Do not get on skin or clothing. Avoid breathing fumes. Read the warning note on the container and MSDS.
- In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and if necessary seek medical attention.

QUALITY CONTROL ANALYSIS

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPrep™ Plant DNA Mini Kit is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

To check before start

Please read the entire instruction manual prior to starting the Protocol Procedure.

Sample Homogenization Step

Sample Type	Action
Adherent Cultured Cells	1. Remove the culture medium from culture dish. 2. Add 1 ml of Wizol™ Reagent per 10 cm^2 of culture dish surface area. Note : Add 1ml of Wizol™ Reagent for a 35mm dish, 3ml for a 60mm dish, 8ml of a 100mm dish. 3. Lyse the cells in the culture dish by pipetting several times.
Suspension Cultured Cells	1. Harvest cell by centrifugation at 300 x g for 5min. Note : 5~10^6 cell from animal, plant or yeast origin, or 1 x 10^7 cell of bacterial origin. 2. Add 1 ml of Wizol™ Reagent to the cell pellet and lyse the cells by pipetting several times. Note : Disruption of some yeast and bacterial cells my require the use of a homogenizer
Tissue	1. Add 1 ml of Wizol™ Reagent to 50 ~ 100mg of tissue sample. 2. Homogenize tissue samples using a homogenizer.
Biological fluids	1. Transfer 300 µl of biological sample to a 1.5 ml tube. Note : Biological sample with high levels of contaminating material (e.g., whole blood) should be diluted 1:1 with RNase-Free water. 2. Add 1 ml of Wizol™ Reagent to liquid sample. 3. Mix well by vortex.

Total RNA Isolation Step

1) Add 200 µl of chloroform to each tube.
2) Shake tube vigorously by hand for 15 sec.
3) Incuatbe at room temperature for 3 min.
4) Centrifuge at 12,000 g at 4ºC for 15 min.
5) Carefully pipet aqueous phase into a 1.5 ml tube. Discard interphase and lower phase.
6) Add 500 µl of Isopropanol.
7) Closed tube and mix by gentle inversion.
8) Incubate at room temperature for 10 min.
9) Centrifuge at 12,000 g at 4ºC for 10 min. Discard supernatant.
10) Wash pellet with 1 ml of 75% ethanol. Vortex briefly.
11) Centrifuge at 7,500 g at 4ºC for 5 min. Discard supernatant
12) Dry pellet on clean wiper for remove the ethanol completely.
13) Add 50~100 µl of DEPC-Water, resuspend RNA by pipetting up and down a few times.
    Note: If having problems resuspending the RNA pellet, we suggest incubation at 60ºC for 10 ~15 min.
14) Determine RNA concentration and quality by spectrophotometry.
    Note: For optimal spectrophotometric measurements, RNA aliquots should be diluted with water or buffer with a basic pH.
    Water with pH < 7.5 falsely decreases the 260/280 ratio.

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TROUBLESHOOTING

Problem	Possiblity	Suggestions
Low yield	• Sample lysis or homogenization was incomplete • DNA/RNA/Protein pellet was not dissolved completely	• Starting material should be reduced and completely dissolved in Wizol™ Reagent. • Increase incubation temperature to 60°C and increase incubation time to 15 minutes. If the pellet is still not dissolved, pipette until it dissolves completely.
Degraded RNA	• Incorrect sample preparation and/or final storage • Incorrect sample storage temperature	• Process or freeze samples immediately after collection. • Extracted RNA should be stored at -70°C.
DNA Contamination	• When removing the aqueous phase, the interephase and/or organic phase were drawn into the pipette	• Leave a small amount of aqueous phase to avoid drawing the interphase and/or organic phase into the pipette.
Low RNA $A_{260/A280}$ (>1.6)	• Volume of Wizol™ Reagent was insufficient for proper sample homogenization • Residual organic solvents (phenol, chloroform) in the RNA rehydrates • Sample not completely homogenized	• Volume of Wizol™ Reagent is sample dependent and should be added according to the sample homogenization specifications. • Be sure not to carry any of the organic phase with the RNA sample • Precipitate the RNA again with ethanol. • Use 1 ml Wizol™ Reagent for up to 100 mg tissue or 10^6 cells. • Be sure to incubate sample for 5 minutes at room temperature after homogenization.

SYMBOL GLOSSARY

REF	Catalogue number		Manufacturer
LOT	Batch code	RUO	Research use only
	Temperature limit		Instructions for use
	Use-by date		

ORDERING INFORMATION

Product	Cat No.	Package
Wizol™ Reagent	W76100	100 ml

Wizbiosolutions Inc.
#1103, 1405, 1406, A-dong, 14, Sagimakgol-ro 45beon-gil, Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea
B237~B242, 14, Galmachi-ro 288beon-gil, Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea

Tel : (82) 70-7603-5066
Fax: (82) 31-624-3066
e-mail: support@wizbiosolution.com
www.wizbiosolution.com