Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

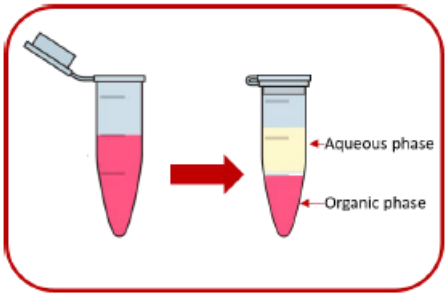
Version: 2
Revision date: 11/04/2023

1. Identification

Product name **Primezol™ Reagent**
2 x 50 mL

Cat. No AN1100

2. Description



PRImeZOL™ Reagent is a ready-to-use reagent for the isolation of total RNA from various biological materials such as animal and plant tissues (rich in polysaccharides and proteoglycans), cell culture and bacterial cells. This procedure is based on the sample lysis in cationic detergent guanidinium thiocyanate (GTC), followed by organic extractions and alcohol precipitation. The biological sample is homogenized or lysed before being separated into three phases: an aqueous phase (upper), an organic phase (lower) and an interphase. The RNA remains in the aqueous phase, and its purification is followed by precipitation in isopropyl alcohol. The chaotropic environment generated by guanidinium thiocyanate rapidly inactivates RNases, protecting RNA from degradation during cell lysis.

After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation.

3. Composition

PRImeZOL™ Reagent contains phenol and the mixture of other reagents to ensure optimal results. Always work in fume hood and wear protective clothing and goggles. Toxic in contact with skin and if swallowed. Causes burns. After contact with skin or eyes, wash immediately with plenty of water. If you feel unwell, seek medical advice (show label where possible).

Item	Quantity
Primezol™ Reagent	2 x 50 mL

4. Features

- ✓ Ready-to-use solution.
- ✓ Quick isolation of high-quality total RNA, DNA and/or protein from a single sample.
- ✓ Performs well with large or small amounts of tissue or cells.
- ✓ Operation time: approximately 60 minutes.
- ✓ RNA resuspension volume: 10–50 µL RNase-free water depending on desired concentration.
- ✓ Compatible sample types: whole blood, buffy coat, cultured animal and plant cells or tissues, bacteria and yeast.

5. Storage specifications

Store at 2-8˚C, protect from light. Do not freeze.

6. Applications

- ✓ Purified RNA is ideal for any downstream application such as RT-PCR, in vitro translation, Northern blotting, RNase protection assays or dot blot hybridization.

Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

- ✓ Purified DNA can be used for PCR and Southern blotting.
- ✓ Purified protein can be used for Western blotting.

RNA obtained is suitable for sensitive downstream applications. For applications requiring complete removal of genomic DNA, DNase treatment is recommended.

7. Quality control

Each lot is tested in accordance with internal procedures.

8. Further information

Product Use Limitations	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Disclaimer	The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

Instructions for RNA isolation

1. Homogenization

Tissues

Homogenize tissue samples in 1 ml of **PRImeZOL™** per 50-100mg of tissue. For small quantities of tissue (1-10 mg), add 800 µl of **PRImeZOL™**.
OPTIONAL: Following homogenization, insoluble material is removed by centrifugation at 12000 x g for 10 minutes at 4˚C. Transfer the cleared homogenate to a fresh tube.

Cells grown on monolayer

Lyse cells directly in a culture dish or flask by adding 1ml of **PRImeZOL™** per 10 cm2 growth area, pipette the cell lysate several times to ensure sufficient cell disruption.

Cells grown in suspension

Pellet cells at 200 x g for 5 minutes at room temperature. Lyse cells with 1 ml of **PRImeZOL™** per 5 x 10^6 cells and pass the lysate several times through a pipette tip. For small quantities of cells (10^2 – 10^6), lyse cells in 800 µl of **PRImeZOL™**.
Note: At this stage, samples can be stored for at least one month at -70˚C.

2. Phase Separation

1. Incubate samples for 5 minutes at room temperature.
2. Add 0.2 ml of chloroform (not supplied) per 1 ml of **PRImeZOL™** used.
3. Cap tubes securely and shake vigorously by hand for 15 seconds.
4. Incubate samples for 3 minutes at room temperature.
5. Centrifuge samples at 12000 x g for 15 minutes (or 2600 x g for 30 minutes) at 4˚C.
6. The sample will separate into a red lower organic layer, an interphase and a colorless upper aqueous layer that contains the RNA.

Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

3. RNA Precipitation

1. Transfer the aqueous phase very carefully, without disturbing the interphase to another tube.
2. Precipitate the RNA by mixing with cold isopropyl alcohol (not supplied). Use 0.5 ml of isopropyl alcohol per 1 ml of **PRImeZOL™** used.
3. Incubate samples for 10 minutes at room temperature.
4. Centrifuge at 12000 x g for 10 minutes (or 2600 x g for 30 minutes) at 4˚C. The RNA precipitate forms a gel-like thigh pellet and may be difficult to see.

4. RNA Wash

1. Carefully remove the supernatant avoiding to touch the pellet.
2. Wash the pellet once with 70% ethanol (not supplied), adding at least 1 ml of ethanol per 1 ml of **PRImeZOL™** used.
3. Vortex samples and centrifuge at 7500 x g for 5 minutes at 4˚C.
4. Carefully remove and discard the ethanol and repeat the washing step.

5. Re-dissolving the RNA

1. Air-dry the pellet and dissolve in PCR grade water by pipetting the solution up and down.
2. Incubate for 10 minutes at 60˚C if necessary.
3. Store RNA at -80˚C.

Instructions for DNA isolation

Following phase separation of the RNA isolation protocol (step 2 on page 3), DNA can be recovered from the interphase (white) and the organic phase (red) after removal of the aqueous phase (colourless) by ethanol precipitation.

1. DNA precipitation

1. Carefully remove any residual aqueous phase layer (upper colourless layer), **This is a critical step for DNA quality!**
2. Add 0.3 ml of absolut ethanol (not supplied) per 1 ml of **PRImeZOL™** used.
3. Mix well by inverting the tube several times.
4. Incubate samples for 5 minutes at room temperature.
5. Centrifuge samples at 2000 x g for 5 minutes at 4˚C.
6. Remove and discard the supernatant.

2. DNA Wash

1. Add 1 ml of 0.1 M sodium citrate in 10% ethanol, pH 8,5 per 1 ml of **PRImeZOL™** used.
2. Incubate the sample for 30 minutes at room temperature. During this incubation, regularly invert the tube gently.
3. Centrifuge at 2000 x g for 5 minutes.
4. Remove and discard the supernatant.
5. Repeat the washing step.
6. Add 1,5 ml of 70% ethanol to the sample per 1 ml of **PRImeZOL™** used.
7. Incubate the sample for 15 minutes at room temperature. During this incubation, regularly invert the tube gently.
8. Centrifuge at 2000 x g for 5 minutes.
9. Remove and discard the supernatant.

3. DNA resuspension

Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

1. Air-dry the pellet for 5 or 10 minutes and dissolve in TE or PCR grade water by pipetting the solution up and down.
2. Incubate for 10 minutes at 55-60˚C if necessary.
3. Centrifuge at 12.000 x g - 16.000 x g for 10 minutes to remove insoluble particles.
4. Transfer the supernatant containing the DNA to a new 1,5-ml microtube.
5. Store DNA at -20˚C.

Instructions for Protein isolation

1. Protein Precipitation

1. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant saved from the DNA precipitation procedure.
2. Mix well by inverting the tube several times.
3. Incubate at room temperature for 10 minutes.
4. In order to precipitate the protein fraction, centrifuge the sample at 14,000g-16,000g for 10 minutes at 4°C.
5. Carefully, remove and discard the supernatant.

2. Washing

1. Add for each 1 mL of PrimeZOL initially used a total of 2 mL of 0.3 M guanidine hydrochloride in 95% ethanol to the protein pellet.
2. Incubate at room temperature for 20 minutes.
   The protein pellet could be stored in this wash/storage for up to one year at -20°C.
3. Centrifuge the sample at 7,000g-8,000g for 5 minutes at 4°C.
4. Remove and discard the supernatant.
5. Repeat this washing procedure twice.

3. Protein Resuspension

1. Add 200 µL if a 1% SDS solution in water to the protein pellet and resuspend by pipetting up and down.
2. Incubate at 50°C for 10 minutes to completely dissolve the protein pellet.
3. Centrifuge at 10,000g for 10 minutes at 4°C to remove debris.
4. Transfer the cleared supernatant to a new 1.5 mL microtube and the protein is ready for downstream applications or storage (at 20°C).