Bedienungsanleitung
Instruction Manual

SERVA BlueZol

Lysis reagent for cells and tissues

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Contents

1. General Information

BlueZol is a ready-to-use reagent for the isolation of total RNA from various biological materials such as animal and p tissues (rich in polysaccharides and proteoglycans), cell culture and bacterial cells.

Using ***BlueZol*** a 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 highly effective RNase inhibitory property of

BlueZol protects the integrity of the RNA during lysis and results in the isolation of high-quality material.

BlueZol contains phenol and the mixture of other reagents to ensure optimal results.

1 ml of ***BlueZol*** is sufficient to isolate total RNA from 1x 10^7 cells or 100 mg of tissue. The isolation method is fast and easy.

1.1. Features

- Quick isolation of high-quality total RNA, DNA and/or protein from a single sample
- Performs well with either large or small amount of tissue or cells
- Ready-to-use solution

1.2. 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
- Purified DNA can be used for PCR and Southern blotting
- Purified protein can be used for Western blotting

1.3. **Storage conditions**: Store at + 2°C to + 8 °C.

2. Protocol for RNA, DNA and Proteins Isolation

2.1. Reagents required (not supplied with *BlueZol*)

- Chloroform
- Isopropyl alcohol (chilled)
- 75 % (v/v) ethanol (in DEPC-treated water)
- DEPC-treated water (SERVA cat. no. 39798) or PCR-grade water
- 100 % ethanol (SERVA cat. no. 39556)
- Resuspension solution for DNA isolation
  (100 mM sodium citrate, 10 % (v/v) ethanol, pH 8.5)
- 8 mM NaOH
- HEPES buffer
- Wash solution for protein isolation
  (300 mM guanidine hydrochloride, 95 % ethanol)
- 1 % (w/v) sodium dodecylsulfate (SDS) solution

2.2. Homogenization

- **Tissue**
  Homogenize tissue samples in 1 ml of ***BlueZol*** per 50 - 100 mg of tissue. For small quantities of tissue (1 - 10 mg), add 800 µl of ***BlueZol***. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed.

- **Plant tissue**
  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 ***BlueZol*** per 10 cm^2 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 ***BlueZol*** per 5x 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 ***BlueZol***.

Note: At this stage, samples can be stored for at least one month at - 80 °C.

2.3. Phase Separation

- Incubate samples for 5 minutes at room temperature.
- Add 0,2 ml of chloroform per 1 ml of ***BlueZol*** used.
- Cap tubes securely and shake vigorously by hand for 15 seconds.
- Incubate samples for 3 minutes at room temperature.
- Centrifuge samples at 12000 x g for 15 minutes (alternatively: 2600 x g for 30 minutes) at 4 °C.
- The sample will separate into a pale yellow organic phase, an interphase (both containing DNA and proteins) and a colorless upper aqueous phase that contains the RNA.

2.4. RNA Isolation

2.4.1. RNA Precipitation

- Transfer the aqueous phase very carefully, without disturbing the interphase to another tube.

  Please note: The remaining organic and interphase can be used for DNA and protein isolation.

- Precipitate the RNA by mixing with cold isopropyl alcohol.
  Use 0.5 ml of isopropyl alcohol per 1 ml of ***BlueZol*** used.
- Incubate the sample for 10 minutes at room temperature.
- Centrifuge at 12000 x g for 10 minutes (or 2600 x g for 30 minutes) at 4 °C.

2.4.2. RNA Wash

- Remove the supernatant.
- Wash the pellet once with 75 % (w/w) ethanol,
  adding at least 1 ml of ethanol per 1 ml of ***BlueZol*** used.
- Vortex the sample and centrifuge at 7500 x g for 5 minutes at 4 °C.

2.4.3. Re-dissolving the RNA

- Air-dry the pellet and dissolve in PCR water or DEPC-treated water by pipetting the solution up and down.
- Incubate for 10 minutes at 60 °C if necessary.
- Store RNA at - 80 °C.

2.5. DNA Isolation

2.5.1. DNA Precipitation

- For subsequent DNA and protein isolation, use the organic and the interphase (see 2.3. and 2.4.) and remove any remaining aqueous phase.
- Precipitate the DNA by addition of 100 % ethanol (0.3 ml per 1 ml of ***BlueZol*** used).
- Mix well by inverting the tube several times and incubate 2 - 3 min.
- Centrifuge at 2000 x g for 5 minutes at 4 °C.
- Remove the supernatant and transfer it to a new tube for further protein isolation, if needed. The supernatant can be stored at - 80 °C.

2.5.2. DNA Wash

- Resuspend the DNA pellet in the resuspension solution (1 ml per 1 ml of ***BlueZol*** used).
- Incubate 30 min at room temperature, mix occasionally by gentle inversion.
- Centrifuge at 2000 x g for 5 minutes at 4 °C.
- Remove and discard the supernatant.
- Repeat this washing step once.
  For large DNA amounts (> 200 µg) it is recommended to repeat it twice.
- Resuspend the DNA pellet in 1.5 – 2 ml 75 % (v/v) ethanol (1 ml per 1 ml of ***BlueZol*** used).
- Incubate 10 - 20 min at room temperature, mix occasionally by gentle inversion.
- Centrifuge at 2000 x g for 5 minutes at 4 °C.
- Remove and discard the supernatant.
- Air-dry the DNA pellet for 5 -10 min.

  Please note:
  Do not dry the pellet by vacuum centrifuge.
  Otherwise it is very hard to solubilize the DNA.

2.5.3. Re-dissolving the DNA

- Resuspend the DNA pellet in 0.3 – 0.6 ml of 8 mM NaOH.
- Centrifuge at 12,000 x g for 10 minutes at 4 °C.
- Transfer the supernatant to a new tube and adjust the pH to 7 - 8 with HEPES.

The DNA sample is now ready for further downstream applications.

2.6. Protein Isolation

2.6.1. Protein Precipitation

- For further protein isolation, transfer the phenol-ethanol supernatant (section 2.5.1.) in a new tube.
- Add isopropyl alcohol (1.5 ml per 1 ml of ***BlueZol*** used).
- Incubate 10 min at room temperature.
- Centrifuge at 12,000 x g for 10 minutes at 4 °C.
- Remove and discard the supernatant.

2.6.2. Protein Wash

- Resuspend the protein pellet in the wash solution (2 ml per 1 ml of ***BlueZol*** used).
- Incubate 20 min at room temperature.
- Centrifuge at 7500 x g for 5 minutes at 4 °C.
- Remove and discard the supernatant.
- Repeat the washing step twice.
- Add 2 ml 100 % ethanol and mix by vortexing briefly.
- Incubate 20 min at room temperature.
- Centrifuge at 7500 x g for 5 minutes at 4 °C.
- Remove and discard the supernatant.
- Air-dry the protein pellet 5 – 10 minutes.

2.6.3. Re-dissolving the protein

- Resuspend the air-dried protein pellet in 200 µl 1 % (w/v) SDS solution.

  Please note:
  To ensure complete resuspension, it is possible to incubate the protein sample at 50 °C.

- Centrifuge at 10,000 x g for 10 minutes at 4 °C.
- Transfer the supernatant to a new tube.

The protein sample is now ready for further downstream applications.

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