



OMNIzol

Cat.# EMR060100

INTRODUCTION

OMNIzol is a ready to use reagent for extraction of total RNA from tissues or isolated cells. This reagent is specifically designed to allow purification of both DNA and proteins, following RNA extraction. To facilitate the purification of RNA, OMNIzol includes a high affinity RNA binding resin. To avoid RNA degradation OMNIzol also contains certified RNase free water. The RNA obtained is undegraded, contains all the different species of RNA, it is free of proteins and DNA.

A special SDS buffer is also included to facilitate solubilization of proteins and to improve their electrophoretic separation.

REAGENTS SUPPLIED

- 1 x 100ml OMNIzol
- 1 x 1ml RNA Binding Resin
- 2 x 1ml Elution Solution (RNase free)
- 1 x 1ml of Protein Loading Buffer

REAGENTS NEEDED BUT NOT PROVIDED

- Chloroform
- Isopropanol
- Ethanol 75%
- Acetone
- RNase free water
- Ethanol 100%
- Sodium citrate 0.1M in ethanol 10%
- Guanidine hydrochloride 0.3M in ethanol 95%
- 1X TE (10mM Tris;1mM EDTA)

STORAGE & STABILITY

Nine months at +4°C.

METHOD FOR RNA PURIFICATION

Homogenization

Use 1ml of OMNIzol for 100mg of tissue or for 2x10⁷ cells. Homogenise the tissue with a few strokes in a glass-tefl on potter.

RNA extraction

1. Add chloroform to each sample in the proportion of 1:10, mix carefully (do not vortex) and keep for 5 minutes in wet ice.
2. Centrifuge at 12.000g for 15 minutes.
3. Collect the upper aqueous phase containing RNA (a cloudy interface including Proteins and lower yellow phase including DNA are also present).

RNA purification

Two methods are recommended according to the starting amount of RNA:

a - Isopropanol precipitation

Indicated for large quantities of RNA.

1. Transfer the upper aqueous phase into a new tube.
2. Add the same amount of cold isopropanol and store in wet ice for 15 minutes.
3. centrifuge at 12.000g at +4°C for 15 minutes.
4. Wash the pellet by adding 1ml of 75% ethanol to each sample.
5. Centrifuge at 8.000g at +4°C for 15 minutes.
6. Resuspend RNA in RNase free water included in the kit.
7. To increase the RNA solubility, if needed, the purified RNA might be incubated for 10 minutes at 55°C.

b - RNA binding resin

This method is recommended to avoid the dispersion of small amounts of RNA (extracted from less than 1x10⁶ cells), to shorten procedure and to inhibit RNA degradation.

1. Add 50µl of resin per 1ml of OMNIzol used for initial extraction
2. incubate for 15 minutes stirring gently
3. Centrifuge for 1 minute in a microfuge (full speed).
4. Wash the pellet adding 1ml of 75% ethanol.
5. Centrifuge for 1 minute in a microfuge (full speed) at room temperature
6. aspirate the supernatant.
7. Repeat the wash with 75% ethanol.
8. Add 1ml acetone and centrifuge for 1 minute in a microfuge (full speed) at room temperature.
9. Aspirate all supernatant and let the pellet dry at 55°C for 5 minutes.
10. Add 100µl of RNase free water (DEPC-treated, autoclaved water) to the pellet.
11. Elute the RNA from the resin by heating at 55°C for 10 minutes.
12. Pellet the resin by spinning for 1 minute in a microfuge (full speed) and collect the supernatant.

METHOD FOR GENOMIC DNA EXTRACTION

DNA is extracted from the interphase and the lower organic phase by ethanol precipitation.

1. All the remaining RNA containing the aqueous phase must be removed very carefully.
2. Add to the organic phase (lower phase) 0.3ml of 100% ethanol per 1ml of OMNIzol initially used.
3. Mix by gentle stirring and stand at room temperature for 3 minutes.
4. Centrifuge the sample at 2000g for 5 minutes at 4°C.
5. The genomic DNA is contained in the pellet.
6. Store the supernatant if further protein extraction is needed.
7. Wash the DNA pellet twice with 0.1M sodium citrate in 10% ethanol. Use at least 1ml of solution for each millilitre of OMNIzol initially used.
8. Incubate the DNA at room temperature for 30 minutes.
9. Centrifuge the sample at 2000g for 5 minutes at 4°C.
10. Perform a final wash adding 2ml of 75% ethanol for each millilitre of OMNIzol initially used, incubate at room temperature for 20 minutes.
11. Centrifuge at 2000g for 5 minutes at +4°C.
12. Let the pellet dry on the bench for 10 minutes.
13. Resuspend the genomic DNA in 1X TE.

METHOD FOR PROTEIN PURIFICATION

Proteins are extracted from the phenol-ethanol supernatant collected after genomic DNA extraction (step 6 of the METHOD FOR GENOMIC DNA EXTRACTION).

1. Add 1.5ml of isopropyl alcohol for each millilitre of OMNIzol initially used.
2. Incubate for 10 minutes at room temperature and centrifuge at 12.000g for 10 minutes.
3. Remove the supernatant and add 2ml of 0.3M guanidine hydrochloride in 95% ethanol for each millilitre of OMNIzol initially used.
4. Incubate 20 minutes at room temperature and centrifuge at 7,500g for 5 minutes at +4°C.

5. Repeat the guanidine hydrochloride wash.
6. Add 2ml of ethanol 100% to the pellet and store at -20°C until needed.
7. If SDS-PAGE has to be performed, centrifuge the sample at 7.500g for 5 minutes at 4°C.
8. Resuspend the pellet in the *Protein Solubilizing Buffer* included in the kit.
9. Incubate for 15 minutes by gentle stirring.
10. Heat at 80°C for 5 minutes.
11. Centrifuge at 12,000g for 10 minutes at room temperature.
12. Load the supernatant onto gel or store at -20°C.

Protein Solubilizing Buffer contains an electrophoretic tracer (bromophenol blue) and glycerol to facilitate sample loading onto gel. *Proteins Solubilizing Buffer* contains SDS which will interfere with protein estimation. If estimation is required, try to resuspend proteins with water containing 0.1% SDS, collect an aliquot for proteins estimation, then add *Protein Solubilizing Buffer* for complete solubilization.

TROUBLESHOOTING

RNA purification

- a. Atypical high band found after electrophoresis: the electrophoretic pattern of the RNA preparation might show an anomalous band, higher than the 28s RNA band. This is **NOT** a genomic DNA contamination, but an artefact due to a very high ethidium bromide concentration in the electrophoretic buffer and/or in the gel. Under these conditions the high concentrated RNA is prone to create high molecular weight concatenamers included in the atypical band present after electrophoresis. To solve this problem just avoid ethidium bromide in the sample and use it just in the gel at a final concentration of 0.4µg/ml.

- b. Low yield: use RNA binding resins to avoid dispersion and degradation of low starting amounts of RNA. Use fresh tissue or cells, keep the sample in ice before extraction. Do not extend the homogenisation procedure beyond 15-20 minutes. Sample volume should not exceed 15% of the volume of OMNIZol added to the sample.
- c. DNA contamination – Low 260/280 ratio: do not aspirate the DNA included in the interface. Be very careful when pipetting the upper phase, leave at least a 2mm layer of aqueous phase. Sample volume should not exceed 15% of the volume of the OMNIZol.

DNA purification

- a. Low yield: insufficient homogenization of the tissue, insufficient solubilization of DNA pellet.
- b. Low 260/280 ratio (>1.7): insufficient DNA washing in citrate solution, too high quantity of starting sample.
- c. RNA contamination: contamination with the upper aqueous phase, insufficient DNA washing in citrate solution.

Proteins Purification

- a. Low yield: insufficient homogenization of the tissue, insufficient solubilization of proteins. Increase the incubation time with *Proteins Solubilizing Solution*.
- b. Smears are present in the gel: presence of DNA. Sonicate the sample. Too high amount of proteins have been loaded on the gel.
- c. Proteins degradation: add protease inhibitor.

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