TheOne™ RNA Reagent

Cat #: RNR-100, RNR-200
Size: 100 ml, 200 ml
Include De-PSG Solution 30 ml, 60 ml

Description:

TheOne Reagent is a single reagent developed for the isolation of **total RNA** from animal and plant tissue, cells and bacteria culture. This reagent can also simultaneously extracted **DNA and proteins** for southern blot, PCR and western blot analysis. The entire procedure for total RNA isolation is an improvement single-step method developed by Chomczynski and Sacchi. After homogenization of sample and chloroform extraction, three phases are formed (aqueous phase, interphase and organic phase). RNA can be precipitated by isopropanol from aqueous phase, DNA can be recovered by ethanol precipitation from interphase, and proteins are precipitated with isopropanol from organic phase. The reagent also includes a bottle of **De-PSG Solution**, which will eliminate polysaccharides and proteoglycans contamination, the RNA will be dissolve easier and purer for RT-PCR and northern blot application.

General Procedure for RNA Isolation :

Materials to be supplied by the user:

- Chloroform, chilled
- Isopropanol, chilled
- 75% Ethanol (in DEPC-treated water or RNase- free water)
- RNA-Defense reagent (cat#) or RNase-free water or 0.5% SDS solution or deionized formamide

A. Homogenization

I. Animal, plant samples and culture cells

a. Animal, plant samples

Homogenize tissues samples (10-100mg) with 1ml of **TheOne Reagent** with a few strokes in a glass-teflon or polytron homogenizer for 15-30 sec until complete lysis. Proceed to RNA isolation section on page 2.

* *Too much sample or incomplete homogenization will cause contamination of genomic DNA and RNA degradation after isolation.*

b. Cells grown in suspension

Sediment cells and discard the culture medium, lyses them by the addition of 1 ml of **TheOne Reagent** to $5x10^6$ – $1x10^7$ of animal and plant cells. Pass the cell lysate several times through a pipette until complete lysis. Proceed to RNA isolation section on page 2.

c. Cells grown in monolayer

Remove and discard the culture fluid. Lyse cells by adding directly to the culture dish or flask 1 ml of **TheOne Reagent** per 10 cm^2 dish **(not according to the cell numbers as suspension cells)**. Pass the cell lysate several times through a pipette until complete lysis. Proceed to RNA

isolation section on page 2.

** Too much sample or incomplete homogenization will cause contamination of genomic DNA and RNA degradation after isolation.*

Notes:

** If using larger samples (>100mg or >10^7 cells), increase* **TheOne Reagent** *proportionally, e.g. 150mg or 1.5x107 cells using 1.5ml* **TheOne Reagent**.

** For small sample 1 to 10 mg or cells 10^2 to 10^4 use only 800µl* **TheOne Reagent.**

** For some samples such as muscles tissue and tuberous parts of plants which contain high amount of protein, fat, polysaccharides or extracellular materials may not be dissolved in homogenate, it is necessary to spin down this insoluble materials by 12,000 x g for 10 min at 4℃. Transfer the supernatant to a new tube for the next step.*

** The homogenate can be stored for one month at -70℃ before isolation.*

II. Bacteria sample

Before starting: freshly prepare lysozyme solution at 4 mg/ml in TE buffer.

a. Transfer bacteria culture (up to 10^8 cells at log phase) to pre-chilled microcentrifuge tube.
b. Spin down the cells at 6,000g for 5 min at 4℃.
c. Discard the supernatant and add 100µl of freshly prepared lysozyme solution to the pellet. Mix well by gently pipetting up and down. Incubate at RT for 10-30 min.
d. Add 1ml **TheOne Reagent** to the cells and pass the cell lysate several times through a pipette until complete lysis.
e. Spin down the insoluble material by 12,000 x g for 10 min at 4℃. Transfer the supernatant to a new tube. Proceed to RNA Isolation section below.

B. RNA Isolation

1. Incubated the homogenate for 5 min at room temperature to completely dissociate the nucleoprotein complex.
2. Add 0.2 ml of **chloroform** per 1 ml of **TheOne Reagent**. Cap and shake vigorously for 15s and incubate them at room temperature for 2-3 min.
3. Centrifuge the samples at 12,000 x g for 15 min at 4℃. After centrifugation, the mixture separates into a yellow phenol chloroform phase, an interphase and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase (with a volume of approximately 0.6 ml per 1 ml **TheOne Reagent**), DNA is in interphase and proteins are in organic phase.
4. Carefully transfer the aqueous phase to a new microcentrifuge tube without disturbing or touching the interphase.

   ** If interphase is disturbing by pipetting or contamination of interphase needs to be minimum, spin the transferred aqueous phase at 12,000 x g for 10 min at 4℃, transfer the supernatant to a new tube.*
5. Add 0.25 ml of **isopropanol** and 0.25 ml **De-PSG Solution** of per 1 ml **TheOne Reagent** and mix gently, store samples for 10 min at RT.

   ** If DNA or Protein isolation is necessary, store the remaining interphase and organic phase solution at -20℃. And proceed to DNA Isolation or Protein Isolation section.*

   ** De-PSG Solution will greatly reduce contamination of polysaccharides and proteoglycans which co-precipitate with RNA..*

   ** Some small RNA< 200 nt RNA (i.e., tRNA,4S RNA) will also be removed, if these RNA are*

interested, skip the addition of De-PSG Solution, in stead adding 0.5 ml isopropanol to precipitate RNA.

** For small sample 1 to 10 mg or cells 10^2 to 10^4, add 5-10μg glycogen before isopropanol precipitation.*

6. Centrifuge the samples at 12,000 x g for 10 min at 4℃ (RNA precipitate forms a white pellet at the bottom of the tube).
7. Carefully remove the supernatant, wash the RNA pellet in 1 ml of **75% ethanol** per 1 ml **TheOne Reagent**. Mix the sample by vortexing and centrifuge at 12,000 x g for 5 min at 4℃.
   ** RNA sample can be stored in 75 % ethanol for one week at 4 ℃ or one year at -20 ℃.*
8. Remove the ethanol and air dry the RNA pellet briefly for 5-10 min.
   Do not dry the RNA pellet by SpeedVac, which will make RNA hard to dissolve.
9. Dissolve RNA in 1X **RNA-Defense reagent** (cat#) or RNase-free water or 0.5% SDS solution or deionized formamide by repetitive
10. pipetting, and incubated for 20 min at 65℃. **Store the samples at -20°C if stored at RNA-Defense reagent or deionized formamide. Store the sample at -70°C if store at RNase-free water or 0.5% SDS solution.**
    ** RNA-defense reagent contains mixture of non-toxin chemicals, which will disrupt RNase and protect RNA from degradation even at RT or 37°C, and stored RNA can be used at many applications including RT-PCR and northern blot.*
    ** RNA store at deionized formamide can also be used in some RT-PCR and northern blot.*
    ** RNA store at 0.5% SDS can only be used in northern blot.*

C. DNA Isolation

Materials to be supplied by the user:

- 100% Ethanol
- 0.1M sodium citrate in 10% ethanol
- 75% Ethanol
- 8 mM NaOH
- 0.1 M or HEPES (free acid), no pH adjustment
- 0.1 M EDTA (pH8.0)

1. For the remaining interphase and organic phase form RNA isolation, remove the aqueous phase completely by carefully pipetting, which reduces the RNA contamination to minimum.
2. Add 0.3 ml **100% ethanol** per 1 ml **TheOne Reagent** to the interphase and organic phase and mix by inverting several times.
3. Incubate the mixture for 2-3 min at RT, then centrifuge at ≤ 2,000g for 5 min at 4℃.
   Too high speed of centrifugation may cause DNA shearing.
4. A white DNA pellet will be at the bottom of the tube, discard the supernatant.
   If protein isolation is desired, transfer the supernatant to a new tube and store at 4 ℃, proceed to Protein Isolation section step 5 on page 4.
5. Add 1ml **0.1M sodium citrate in 10% ethanol** per 1 ml **TheOne Reagent** to the DNA pellet.
6. Incubate the solution at RT for 30 min, vortex several times during incubation to remove the phenol from pellet, then centrifuge at ≤ 2,000g for 5 min at 4℃.
7. Wash one or two more times with **0.1M Sodium Citrate in 10% ethanol** as procedure 5-6.
8. After above two or three washes, add 1.5 ml **75% ethanol** and incubate at RT for 10 min and

vortex 2-3 time during incubation, then centrifuge at ≤ 2,000g for 5 min at 4℃.

9. Remove the supernatant, air dry the pellet for 5-10 min. Add 300-600µl of **8 mM NaOH** to dissolve the DNA pellet (DNA will not be dissolved in water or TE buffer), incubate at 60℃ and flicking the tube from time to time to facilitate DNA to dissolve.
   ** Do not dry the DNA pellet by SpeedVac, which will make DNA hard to dissolve.*
   ** DNA in 8 mM NaOH can only be stored overnight at 4 ℃.*
   ** DNA may contain insoluble material, remove it by spin at 12,000 g for 10 min and transfer the supernatant to a new tube.*
10. Adjust the pH of DNA solution to pH 7.5-8.0 by adding 12µl of **0.1 M HEPES** and 1µl of **0.1 M EDTA** per 100µl of DNA solution. Now the DNA can be stored at -20℃ for months and ready for PCR or restriction digestion.

D. Protein Isolation

Materials to be supplied by the user:

- Isopropanol
- 0.3M guanidine hydrochloride in 95% ethanol
- Ethanol
- 1% SDS

1. For the remaining interphase and organic phase form RNA isolation, remove the aqueous phase completely by carefully pipetting, which reduces the RNA contamination to minimum.
2. Add 0.3 ml **100% ethanol** per 1 ml **TheOne Reagent** to the interphase and organic phase and mix by inverting several times.
3. Incubate the mixture for 2-3 min at RT, then centrifuge at ≤ 2,000g for 5 min at 4℃.
4. Transfer the supernatant to a new tube and store at 4℃.
5. For the phenol-ethanol supernatant, add 1.5 ml isopropanol to the solution per 1 ml **TheOne Reagent** (if using 1.5 ml tube, separate the phenol- ethanol supernatant into two 1.5 ml tubes, and add 0.75 ml isopropanol to each tube).
6. Incubate the mixture for 10 min at RT, and then spin at 12,000 x g for 10 min at 4℃.
7. Remove the supernatant and wash the pellet by 2 ml of **0.3M guanidine hydrochloride in 95% ethanol** per 1 ml **TheOne Reagent** (if using 1.5 ml tube, add 1ml **0.3M guanidine hydrochloride in 95% ethanol** to each tube).
8. Incubate the solution at RT for 20 min, vortex several times during incubation to remove the phenol from pellet, then centrifuge at 7,500g for 5 min at 4℃.
9. Wash two more time as above, and discard the supernatant.
10. Add 2 ml **ethanol** to the pellet (if using 1.5 ml tube, add 1ml **ethanol** to each tube), incubate for 20 min at RT, then centrifuge at 7,500g for 5 min at 4℃.
11. Air dry the pellet for 5-10 min, dissolve the protein pellet in 100-200µl of **1% SDS** at 50℃. Store the protein sample at -20℃ and is ready for use in PAGE /Western blotting.
    Solution may contain insoluble material, remove it by spin at 12,000 g for 10 min and transfer the supernatant to a new tube.

BIONOVAS Biotechnology Co., Ltd.
647-808-8236
No. 194 kingsdale Ave, Toronto. Ontario. M2N3W9