ORAgene•RNA

Oragene®•RNA Purification Protocol using TRI Reagent® LS (Trizol)

Introduction

TRI Reagent®LS is a quick and convenient reagent for use in the isolation of RNA from liquid samples of human origin. The procedure is an adaptation of the single step method reported by Chomczynski and Sacchi for total RNA isolation. This protocol adapts the TRI Reagent LS procedure for use with Oragene®•RNA/saliva samples. When an Oragene•RNA/saliva sample is mixed with TRI Reagent, and chloroform is added, the mixture separates into 3 phases: an aqueous phase containing RNA, the interphase containing degraded DNA, and an organic phase containing degraded protein.

Safety and Environmental Information

- The use of safety gloves is required to avoid sample contamination.
- TRI Reagent LS and chloroform should always be used in a fume hood.

Equipment and Reagents

Equipment

- Microcentrifuge (capable of speeds > 13,000 x g)
- Optional: microtube holder for vortex mixer (e.g., VWR cat. # 12620-874)
- Optional: fumehood

Reagents

- TRI Reagent LS (Sigma Aldrich cat. # T-3934)
- Chloroform (CHCl3)
- Cold ethanol solutions: 75% and 95% (v/v) (-20°C)
- 5 M Sodium acetate (NaOAc) pH 5.5
- DEPC (diethylpyrocarbonate)-treated water or RNase-free water

Part I – Laboratory preparation and storage of Oragene•RNA/saliva samples

Step	Notes
1-1. When samples are received in the lab, shake very vigorously for 8 seconds or longer.	• Thorough mixing of the Oragene•RNA solution and saliva is necessary to ensure maximum RNA recovery and stability.
1-2. Incubate entire sample in the original vial at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.	• The entire sample must be heated at 50°C prior to any subsequent purification step. • Once heated at 50°C, samples may be stored at room temperature for up to 8 weeks (from date of collection), or stored indefinitely at -20°C.

Part II – Purification of an aliquot of saliva/Oragene•RNA sample using TRI Reagent LS

2-1. Transfer a 250 µL aliquot to a 1.5 mL micro-centrifuge tube.	• If the sample has been stored at -20°C, thaw at 37°C. • Any remaining material in the Oragene•RNA container can be stored for up to 8 weeks at room temperature (from date of collection), or stored indefinitely at -20°C.
2-2. Incubate the aliquot at 90°C for 15 minutes, then cool to room temperature.	• Care should be taken not to exceed 90°C. A water bath is the preferred method of heating. A heating block can be used but temperature should be carefully monitored.
2-3. Add 750 µL of TRI Reagent LS, then vortex vigorously for a minimum of 2 minutes.	• TRI Reagent LS should be used in a fume hood. • Vigorous vortexing is essential for proper isolation of RNA. • A vortex "microtube holder" can be used for vortexing multiple tubes for up to 5 minutes.
2-4. Incubate sample for 5 minutes at room temperature.	
2-5. Add 200 µL of chloroform, then vortex vigorously for 2 minutes.	• A vortex "microtube holder" can be used for vortexing multiple tubes.
2-6. Centrifuge sample for 10 minutes at maximum speed (>13,000 x g).	
2-7. Transfer 450 µL of the aqueous phase to a fresh tube.	• Ensure that only the top aqueous phase, which contains the RNA, is transferred.
2-8. Add 200 µL of chloroform to the aqueous phase, then vortex vigorously for 30 seconds.	
2-9. Centrifuge sample for 10 minutes at maximum speed (>13,000 x g).	
2-10. Transfer 350 µL of the aqueous phase to a fresh tube.	• Ensure that only the top aqueous phase containing RNA is transferred.
2-11. Add 10.5 µL of 5 M NaOAc (150 mM final), then vortex for 15 seconds.	• NaOAc is needed for efficient ethanol precipitation of RNA.
2-12. Add 2 volumes (700 µL) of cold 95% ethanol.	
2-13. Incubate sample for a minimum of 20 minutes at -20°C.	• Using 2 volumes of cold 95% ethanol and incubation at -20°C ensures maximum precipitation and recovery of RNA.
2-14. Centrifuge sample for 3 minutes at maximum speed (>13,000 x g).	
2-15. Carefully remove and discard the supernatant.	• The RNA will be found in a compact clear pellet at the bottom of the tube.
2-16. Wash the pellet with 1 mL of cold 75% ethanol.	• To avoid disturbing the pellet, add the ethanol to the tube gently. Let the sample stand at room temperature for 1 minute.
2-17. Carefully remove and discard the supernatant, without disturbing the pellet.	• Complete removal of this supernatant is essential to prevent inhibition of downstream applications. • To completely remove the ethanol, the tube can be centrifuged for 8 seconds; any residual ethanol can then be removed using a pipette.
2-18. Air-dry the pellet for 5 minutes.	• This is to ensure complete removal of the ethanol.
2-19. Dissolve RNA in 100 µL DEPC-treated water or RNase-Free water.	

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