

BIGTH Total RNA Purification kit

General Guidelines

- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipette tips and tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified material.
- Always use proper microbiological aseptic techniques when working with RNA.
- Use clear polypropylene disposable tubes when working with BIGTH RNA Isolation kit. Do not use tubes that leak or crack.

Instructions for total RNA Purification

Caution:

When working with BIGTH RNA Lysis Buffer use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

I. Obtaining cell lysate.

A. From tissue culture cells and animal tissue.

Tissue culture cells:

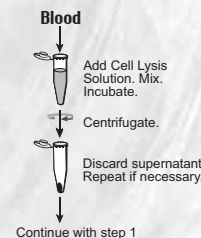
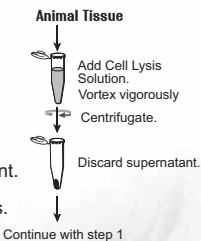
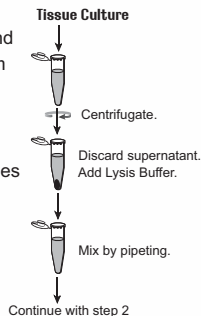
1. a) Suspension cells: centrifuge 200µl (10⁶ cells) of suspension at 14,000 rpm. Discard supernatant.
- b) Attached cells: trypsinize cells using standard methods and proceed as for suspension cultures.
2. Add 150µl of BIGTH RNA Lysis Buffer.
3. Mix by pipeting until complete dissolution of pellet.
4. Continue with step 2 of the protocol "Total RNA purification".

Animal tissue:

1. Add 500µl of Cell Lysis Solution to 10-20mg of sample.
2. Vortex vigorously.
3. Centrifuge 1 minute at 14,000 rpm.
4. Discard supernatant.
5. Continue with step 1 of the protocol "Total RNA purification".

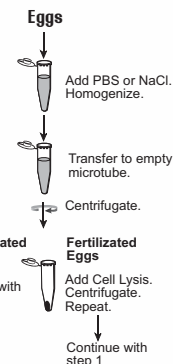
B. From Whole Blood.

1. Add 500µl of Cell Lysis Solution to 200µl of whole blood. Mix by inversion.
2. Incubate for 5 minutes at room temperature.
3. Centrifuge 1 minute at 14,000 rpm.
4. Discard supernatant.
5. The pellet must be completely white. Otherwise repeat steps 1 to 4.
6. Continue with step 1 of the protocol "Total RNA purification".



C. From eggs.

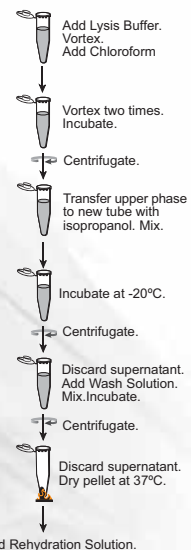
1. Put a representative sample of 5 eggs in a 13 mL sterile polypropylene tube.
2. Add 5mL of PBS or NaCl 0.9% (w/v)
3. Homogenize.
4. Transfer 500 µl of mixture to a clean microtube.
5. Centrifuge 1 minute at 14,000 rpm.
6. Discard supernatant.
7. a) **Fertilized eggs:** add 500 µl de Cell Lysis Solution, mix by inversion and continue with step 8.
- b) **Unfertilized eggs:** continue with step 1 of the protocol "Total RNA purification".
8. Incubate for 5 minutes at room temperature.
9. Centrifuge 1 minute at 14,000 rpm.
10. Discard supernatant.
11. Repeat steps 7. a) to 10.
12. Continue with step 1 of the protocol "Total RNA purification".



II. Total RNA purification.

1. Add 150 µl of BIGTH RNA Lysis Buffer to lysate. Vortex until complete dissolution of the pellet.
2. Add 40 µl of chloroform.
3. Vortex two times for 30 seconds.
4. Incubate 30 seconds at room temperature.
5. Centrifuge the sample at 14,000 rpm for 15 minutes at room temperature.
6. Transfer ~60µl of the colorless, upper phase containing the RNA to a tube containing 60 µl of isopropyl alcohol.
7. Mix by inversion.
8. Incubate 10 minutes at -20°C.
9. Centrifuge at 14,000 rpm for 10 minutes at room temperature.
10. Carefully discard the supernatant, leaving approximately 5 µl of fluid in the tube.
11. Add 150 µl of BIGTH RNA Wash Solution and mix by inversion.
12. Incubate 1 minute at room temperature.
13. Centrifuge at 14,000 rpm for 5 minutes.
14. Discard supernatant.
15. Dry pellet 5 minutes at 37°C.
16. Add 6 µl of BIGTH RNA Rehydration Solution.

Total RNA Purification



NOTE: The reagents in the kit, are designed for 50 RNA purifications, from samples of 200µl of whole blood.

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