BioChain®
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User's Manual and Instructions

Total RNA Extraction Kit

Catalog Number: K2014005

Applications

- Total RNA Isolation

Description

Biochain's Total RNA Extraction kit is a convenient tool for isolating high quality Total RNAs. The isolated Total RNAs can be used for mRNA isolation, probe generation, RT-PCR, Northern blot analysis, primer extension, RNA protection assay, and In vitro translation etc. The kit contains enough reagents for isolating Total RNAs from 5 grams tissue and 10 g rams cells.

Quality Control

The quality and purity of isolated total RNA were tested by spectrophotometer. $A_{260/280}$ is between 1.8 and 2.0 (detected in 10 mM Tris-Cl, pH 7.5). The integrity of the RNA is examined by visual inspection for the presence of intact bands of 18s and 28s ribosomal RNA when electrophoreses on a denaturing agarose gel.

Kit Components

Item	Amount	Storage
1. Solution 1	50 ml	4°C
2. Phenol A	50 ml	4°C
3. Solution 2	6 ml	RT
4. Solution 3	50 ml	RT*
5. DEPC H_2O/0.1mM EDTA	50 ml	RT

*If precipitate formed in solution 3, place the bottle at 65°C water bath to dissolve it before use.

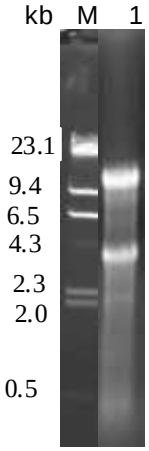

Fig. The image of Total RNA from monkey colon tissue on 1% agarose gel

Items not supplied: 1. Isopropanol; 2. 100% Ethanol; 3. 70% Ethanol; 4. Chloroform; 5. RNase-free DNase I

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Recommended Protocol:

1. Weight certain amount of tissue, crush tissue by hammer, and put it into a new 50 ml centrifuge tube. Stand the tube on ice. Don't let tissue thaw when handling it.
2. Add 10 ml solution 1 per gram tissue, or 5 ml solution 1 per gram cells and blood, homogenize until no visible tissue mass. Add equal volume solution 2 per gram tissue or cells, mix well
3. Add 10 ml Phenol A (5 ml for cells and blood) per gram tissue, shake vigorously for 1 minute
4. Add 4 ml (2 ml for cells) Chloroform per gram tissue, shake vigorously to mix
5. Place tube on ice for 15 minutes
6. Centrifuge the tube at 18,000 g for 15 minutes at 4°C
7. Transfer the supernatant to a new 50 ml centrifuge tube
8. Add 1 volume of isopropanol to the supernatant from step 7, and mix well
9. Store at –20°C for at least one hour
10. Centrifuge the tube at 18,000 g for 15 minutes at 4°C
11. Discard the supernatant and dissolve RNA pellet in DEPC H_2O/0.1 mM EDTA. If you need to do DNase treatment, go for the following steps.
12. Adjust RNA concentration to 0.3 μg/μl by DEPC H_2O/0.1 mM EDTA
13. Add ½ volume of solution 3 in the RNA solution in step 11, mix well
14. Store at –20°C for 4 hours or over night
15. Centrifuge the tube at 18,000 g for 15 minutes at 4°C.
16. Wash the RNA pellet by 70% ethanol. Use 10 ml 70% ethanol per gram tissue
17. Centrifuge at 18,000 g for 15 minutes at 4°C
18. Discard supernatant, dissolve the RNA in DEPC H_2O/0.1 mM EDTA
19. Store the RNA at –70°C

Trouble shooting

1. RNA degradation
   Do not let tissue thaw when handling it. Perform RNA isolation steps at low temperature. Always wear gloves when perform RNA isolation and analysis.
2. Low yield
   Homogenize tissue completely. Collect at least 80% of supernatant for RNA and DNA isolation.
3. Difficult to dissolve RNA pellet
   Do not dry RNA pellet over
4. Genomic DNA contamination
   Treat the RNA with RNase-free DNase I.