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User's Manual and Instructions

Cartilage RNA Isolation Kit

Catalog Number: K2031010

Features

- Isolate RNA from cartilage tissues.

Applications

- Isolation total RNA not only from cartilage tissues but also from other kind of tissues.

Description

Many researchers are studying gene expression in cartilage, and it is a challenge to get high quality RNA from cartilage tissues. This kit provides a convenient and efficient way for isolation of cartilage RNA. The isolated RNA can be used for mRNA isolation, probe generation, RT-PCR, Northern blot analysis, primer extension, RNA protection assay, and In vitro translation.

Quality Control

A representative kit from the same lot is randomly selected for isolation of RNA. 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. Solution 4	14 ml	RT*
6. DEPC H_2O/0.1mM EDTA	50 ml	RT

*If precipitate formed in solution 4, place the bottle at 65°C water bath to dissolve it before use.

Items not supplied:

1. Isopropanol
2. 100% Ethanol
3. 70% Ethanol

4. Chloroform

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, homogenize until no visible tissue mass. Add 1 ml solution 2 per gram tissue, mix well
3. Add 10 ml Phenol A per gram tissue, shake vigorously for 1 minute
4. Add 4 ml 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 for RNA isolation.
8. Add 1 volume of Solution 3 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, dissolve the RNA pellet in DEPC H_2O/0.1 mM EDTA, and adjust RNA to 0.3 μg/ul by DEPC H_2O/0.1 mM EDTA
12. Add ½ volume of solution 4 in the RNA solution in step 11, mix well
13. Store at –20°C for over night
14. Centrifuge the tube at 18,000 g for 15 minutes at 4°C.
15. Wash the RNA pellet by 70% ethanol. Use 10 ml 70% ethanol per gram tissue
16. Centrifuge at 18,000 g for 15 minutes at 4°C
17. Discard supernatant, dissolve the RNA in DEPC H_2O/0.1 mM EDTA
18. 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 completely