User's Manual

Product: **Dr.P kit**-Isolation of RNA, DNA and Protein from the same piece of tissue simultaneously

Catalog Number: K2021010

Features

- Isolate RNA, DNA and Protein from the same piece of tissue simultaneously
- Isolate RNA, DNA and Protein from small or large samples
- Isolated RNA, DNA and Protein from cells or solid tissue

Applications

- Dr.P kit can be used for isolation of RNA, DNA and Protein from the same piece of tissue

Description

In order to study the function of DNA, RNA, protein and their relationship, it is very important to get RNA, DNA and protein from the same piece of tissue. Dr.P kit, a patent pending technology, provides a convenient and efficient system for isolation of RNA, DNA, and protein from the same piece of tissue simultaneously. 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. The isolated DNA can be used for PCR amplification. And the isolated protein can be used for Western Analysis.

Quality Control

A representative kit from the same lot is randomly selected for isolation of RNA, DNA and protein. 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. Beta-actin gene, 838 bp fragment, is successfully amplified from the isolated genomic DNA. And the isolated protein's quality is ensured by Western blotting analysis with anti-GAPDH antibody.

Kit Components

Item	Amount	Storage
1. Solution 1	50 ml	4°C
2. Water saturated phenol	50 ml	4°C
3. Solution 2	6 ml	RT
4. Solution 3	50 ml	RT*
5. 0.5% SDS	25 ml	RT
6. DEPC H_2O/0.1mM EDTA	50 ml	RT
7. TE buffer	5 ml	RT
8. RNAase (10 μg/μl)	14 μl	-20°C

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

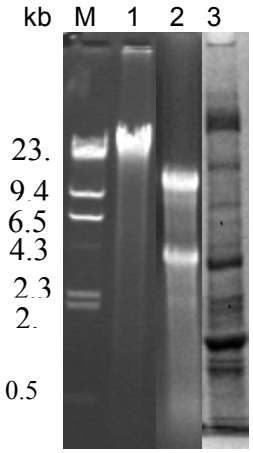

Fig. 5.1 The image of Dr. P product set from monkey colon tissues. Lane 1: Dr. P genomic DNA on agarose gel; Lane 2: Dr. P Total RNA on agarose gel; Lane 3: Dr. P protein on SDS-PAGE gel.

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
   Important: high speed and long time homogenization will cut genomic DNA into small parts. If large size genomic DNA desired, grinding tissue in liquid nitrogen by teflon (or glass) pestle before solution 1 is added.
3. Add 10 ml water saturated phenol 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 and DNA isolation. **Save the organic phase for protein preparation**
8. **RNA isolation**
9. Add 1 volume of isopropanol to the supernatant from step 7, and mix well
10. Store at –20°C for at least one hour
11. Centrifuge the tube at 18,000 g for 15 minutes at 4°C
12. Discard the supernatant, dissolve the RNA and DNA pellet in DEPC H_2O/0.1 mM EDTA, and adjust RNA and DNA concentration to 0.3 μg/ul by DEPC H_2O/0.1 mM EDTA
13. Add ½ volume of solution 3 in the DNA and RNA solution in step 12, mix well
14. Store at –20°C for over night
15. Centrifuge the tube at 18,000 g for 15 minutes at 4°C. **Save supernatant for DNA isolation**
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
20. **DNA isolation**
21. Add 1 volume of isopropanol to the saved supernatant from step 15
22. Store at –20°C for at least 1 hour
23. Centrifuge at 18,000 g for 15 minutes at 4°C
24. Wash the DNA pellet by 70% ethanol. Use 10 ml 70% ethanol per gram tissue
25. Centrifuge at 18,000 g for 15 minutes at 4°C to collect DNA if necessary
26. Discard supernatant. And dissolve the DNA pellet in TE buffer
27. Store the DNA at –20°C
28. **Extraction of protein**
29. Discard the aqueous phase and the inter-phase as much as possible from **the organic phase** at step 7
30. Add 2 volume of isopropanol to the organic phase, mix well
31. Store at room temperature for 5 minutes
32. Centrifuge at 1,800 g for 5 minutes at room temperature
33. Discard the supernatant. Cut the protein pellet into small pieces
34. Put the pellet back to a new 50 ml tube
35. Soak the pellet with 2 volume of 100% ethanol for 15 minutes, centrifuge at 1,800 g for 5 minutes
36. Repeat step 35, and discard supernatant
37. Carefully remove all remained ethanol with a pippet and dry the pellet
38. To elute the protein from the pellet, add 3-5 ml 0.5% SDS per gram tissue to the tube, place the tube at 65°C for 30 minutes.
39. Centrifuge at 1,800 g for 5 minutes, collect the supernatant, and discard the pellet
40. Measure protein concentration
41. Store at 4°C or –20°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. Genomic DNA contaminated by RNA
   Remove RNA by RNase treatment according to standard protocol
4. Difficult to dissolve RNA pellet
   Do not dry RNA pellet over
5. Difficult to dissolve protein pellet
   Add more 0.5% SDS, or place at 65°C longer.