Extracting total RNA from

FFPE tissue samples

Introduction

In the past decade, modern molecular biology technology has been widely used in various fields of human disease research, accumulating new data for understanding the changes in nucleic acid under pathological conditions. Paraffin embedded tissue is a classic method for preserving biological samples. The large amount of paraffin embedded tissue stored in the archives of the hospital pathology department is a reliable source of material for molecular biology research. The extraction of DNA/RNA from paraffin embedded tissues has expanded the clinical application of molecular biology techniques. If high-quality DNA/RNA can be successfully extracted from FFPE samples, it can meet the needs of certain tumor molecular biology research, end the history of DNA/RNA research relying on fresh or frozen tissues and cells, and can be widely applied in retrospective studies of large cases. It has important value in exploring the molecular mechanisms of tumor occurrence, diagnosis and differential diagnosis research, as well as patient prognosis evaluation. Extracting RNA from paraffin tissue poses great challenges. Samples can cause damage to nucleic acids during fixation, embedding, and storage. Nucleic acid can also be modified or encapsulated due to protein cross-linking or protein nucleic acid cross-linking. In addition, RNA can also be fragmented and chemically modified. Magen's HiPure FFPE RNA Kits use silica gel column purification technology and a unique solution system to efficiently extract high-purity RNA from FFPE tissue samples. More detailed operating procedures and high-temperature modification remove treatment can maximize the yield of RNA. This product series includes:

Cat. No.	Name	Product	Method
R4143	HiPure FFPE RNA Kit	Total RNA	Column
R4144	HiPure FFPE RNA Plus Kit	Total RNA	Column
R4313	HiPure FFPE miRNA Kit	Micro RNA	Column
IVD3022	MagPure FFPE RNA Kit	Total RNA	Magnetic

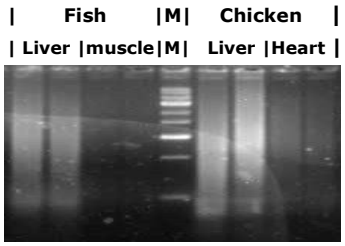
Experiment Data

1. HiPure FFPE RNA Kit

1.1. RNA Production and Electrophoresis Results

Take 9mg paraffin embedded tissue grass carp liver, grass carp muscle, chicken liver, and chicken heart fish liver samples (one year), extract using HiPure FFPE RNA Kit. Take the purified RNA and measure the OD value with Nanodrop 2000, then analyze by 2% agarose gel electrophoresis.

M：CL5000 DNA Marker。

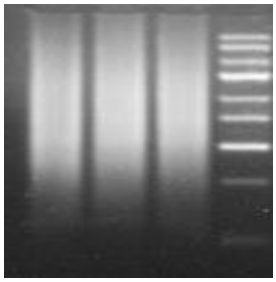


OD260/OD280 is about 2.0, and OD260/OD230 is between 1.8 and 2.0, indicating high RNA purity.

From the yield, it can be seen that the RNA yield is also very high.

Sample	Conc. ng/μl	260/280	260/230	Yield/μg
Fish Liver	372.1	1.88	1.13	14.8
	574.7	1.78	0.94	22.9
Fish Muscle	39.1	1.69	0.36	1.5
	44.7	1.75	0.39	1.7
Chicken Liver	532.3	1.96	1.38	21.2
	627.7	1.92	1.21	25.1
Chicken Heart	70.1	1.84	0.58	2.804
	71.8	1.84	0.57	2.8

1.2. DNA contamination in paraffin RNA



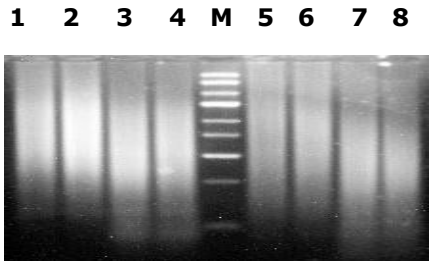
Take the purified paraffin embedded chicken liver RNA, digest with DNase I, remove DNA contamination, and analyze by electrophoresis. As shown in the figure, there was no significant change in the electrophoresis band pattern of chicken liver RNA without or after DNase I treatment, indicating that there was little DNA contamination in the RNA obtained by HiPure FFPE RNA Kit. This is mainly because DNA is cross-linked by histones, and during short digestion, DNA has not yet fully dissociated. HiPure FFPE RNA Plus Kit adopts a unique DNase digestion process by directly adding DNase activation solution to the digestion solution. This solution can shield the influence of SDS and protease on DNase, allowing DNase in the digestion solution to digest and remove DNA, thus it can obtain DNA free RNA.

2. HiPure FFPE miRNA Kit

Take 10mg paraffin embedded tissue chicken liver and fish liver samples (one year) and extract using HiPure FFPE miRNA Kit. During extraction, different concentrations of ethanol (0.5x and 1.5x) were

added to achieve the screening of total RNA and micro RNA. Take the purified RNA, use Nanodrop 2000 to measure its OD value and analyze by 2% agarose gel electrophoresis.

M：CL5000 DNA Marker。



（micro RNA）

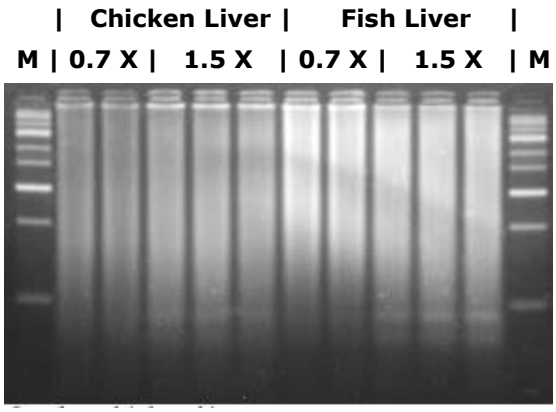
From the OD value, it can be seen that OD260/OD280 is about 2.0, and OD260/OD230 is between 1.8 and 2.0, indicating high RNA purity. From the yield, it can be seen that the RNA yield is also very high. From electrophoresis, it can be seen that the separation of large molecule RNA and micro RNA can be easily achieved by changing the concentration of ethanol. When ethanol reaches 1.5x, there are still many bands of micro RNA below 100bp. If ethanol is 0.5x, there are no bright bands near 100bp, indicating that micro RNA is removed while large molecule RNA is enriched.

Sample ID			Conc. ng/μl	260/280	260/230	Yield μg
鸡肝	Total RNA	1	534.1	2.07	2.07	26.7
	Micro RNA	2	598.3	2.06	2.11	29.9
		3	612.1	2.05	2.1	30.6
		4	599.4	2.06	2.11	29.9
鱼肝	Total RNA	5	295.7	2.03	1.82	14.7
	Micro RNA	6	318.8	2.03	1.83	15.9
		7	384	2.02	1.81	19.2
		8	377.3	2.03	1.81	18.8

3. MagPure FFPE RNA Kit

Take 5mg paraffin embedded chicken liver and fish liver samples (one year) and extract using MagPure FFPE RNA Kit. During extraction, different concentrations of isopropanol (0.7x and 1.5x) were added to achieve the screening of total RNA and micro RNA. Take the purified RNA, use Nanodrop 2000 to measure its OD value and analyze by 2% agarose gel electrophoresis.

M：CL5000 DNA Marker。



From the OD value, it can be seen that the OD260/OD280 is about 2.0, indicating high RNA purity. From the yield, it can be seen that the RNA yield is also very high. From electrophoresis, it can be seen that the separation of large molecule RNA and small molecule RNA can be easily achieved by changing the concentration of ethanol. When the amount of isopropanol reaches 1.5x, there are still many bands of small molecule RNA below 100bp. If the amount of isopropanol is 0.7x, the bright bands around 100bp are not obvious, indicating that micro RNA is removed while large molecule RNA is enriched.

Sample ID		Conc. ng/μl	260/280	260/230	Yield μg
Chicken liver	0.7V	318.1	2.02	1.29	9.5
		409.7	2.07	1.79	12.2
	1.5V	654.5	1.96	1.21	19.6
		691.4	2.06	1.62	20.7
Fish Liver	0.7V	277.4	1.86	0.95	8.3
		195.2	2.06	1.43	5.8
	1.5V	371.1	1.83	0.87	11.1
		255.7	2.07	1.08	7.6