

## Universal miRNA extraction

### --D4130 HiPure Universal miRNA Kit

#### Introduction

At present, commonly used RNA purification methods are mainly suitable for large molecule RNA (>200nt). Small molecule RNA, especially miRNA less than 50nt, often suffers loss or low recovery efficiency as well as unenrichment due to the inability to separate large and small RNA molecules, which affects downstream applications. Magen's Universal miRNA Kit uses MagZol Reagent extraction technology and silica gel column purification technology to efficiently enrich small molecule RNA (<200nt) from biological samples such as 30-50mg animal tissue,  $1 \times 10^7$  cultured cells, and 50-100mg plant samples. The enriched small molecule RNA contains almost no contamination of large molecule RNA and genomic DNA, and can be directly used for applications such as RT-PCR, chip analysis, Northern hybridization, etc. In addition, the kit can also purify its byproduct, large molecular RNA (>200nt). The obtained large molecular RNA can be directly used for expression analysis such as RT-PCR and Northern blotting.

Sample Type	Sample amount	miRNA Yield	Large molecule RNA Yield
Animal tissue	<50mg	<30 $\mu$ g	<150 $\mu$ g
Plant tissue	<100mg	<30 $\mu$ g	<150 $\mu$ g
Cultured cell	< $1 \times 10^7$	<30 $\mu$ g	<150 $\mu$ g
Bacteria	< $5 \times 10^8$	<30 $\mu$ g	<150 $\mu$ g
Yeast	< $2 \times 10^7$	<30 $\mu$ g	<150 $\mu$ g

#### Result

##### 1. Extraction effect of large and small molecules from animal tissue samples

Take 50mg chicken tissue samples and extract large and small molecule RNA using the HiPure Universal miRNA Kit

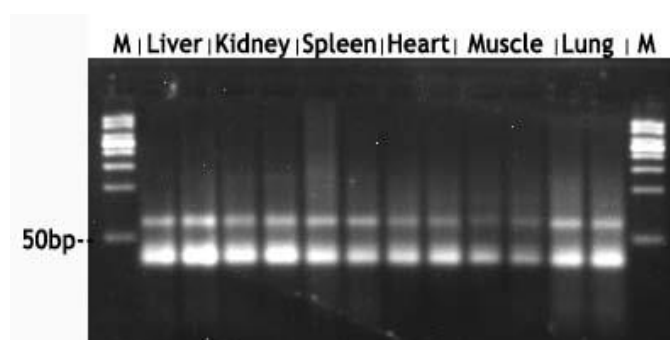
###### 1.1 Extraction effect of small molecule RNA

After extraction, small molecule RNA was obtained and its OD value was measured using Nanodrop 2000. The band pattern of small molecule RNA was analyzed by 3% agarose electrophoresis.

Sample	Conc. $\mu$ g/ $\mu$ l	A260	A280	260/280	Yield $\mu$ g
Liver	0.4888	12.22	6.5	1.88	29.3
Liver	0.4334	10.835	5.315	2.04	26.0
Kidney	0.2019	5.048	2.52	2.00	12.1
Kidney	0.2296	5.74	2.851	2.01	13.7
Spleen	0.3211	8.027	4.018	2.00	19.2
Spleen	0.2175	5.438	2.703	2.01	13.0
Heart	0.1459	3.647	1.806	2.02	8.7
Heart	0.1414	3.535	1.774	1.99	8.4
Muscle	0.1896	4.74	2.429	1.95	11.3
Muscle	0.1415	3.536	1.883	1.88	8.4
Lung	0.2302	5.754	2.858	2.01	13.8
Lung	0.2077	5.192	2.583	2.01	12.4

According to the data, OD<sub>260/280</sub> is between 1.9~2.1, indicating high purity of small molecule RNA. According to the yield, the small molecule RNA content in 50mg liver is about 26-29  $\mu$ g, while that in muscle is also as high as 11 $\mu$ g, indicating a high yield of small molecule RNA.

Take ~1 $\mu$ g small molecule RNA and analyze by 3% agarose gel electrophoresis. The results are as follows. As shown in the figure, there is no contamination of large molecule RNA in the small molecule RNA obtained by this method. According to the DNA marker, small molecule RNA is mainly located in fragments below 50bp. This indicates that the kit can efficiently enrich small molecule RNA.



## 1.2 Extraction effect of large molecule RNA

After extraction, the large RNA was obtained and its OD value was measured using Nanodrop 2000. The results show that the yield of large molecule RNA is high and the purity is also ideal.

Sample	Conc. $\mu\text{g}/\mu\text{l}$	260/280	260/230	Yield $\mu\text{g}$
Liver	0.4231	2.11	1.94	169.2
Liver	0.4538	2.07	1.98	181.5
Kidney	0.2599	2.08	1.99	103.9
Kidney	0.2573	2.06	1.99	103.7
Spleen	0.6382	2.11	2.28	127.6
Spleen	0.6383	2.11	2.06	127.7
Heart	0.3654	2.13	1.96	73.1
Heart	0.3342	2.11	2.41	66.8
Muscle	0.0334	2.1	1.99	6.7
Muscle	0.0558	1.82	2.57	11.2
Lung	0.1738	2.09	2.02	34.8
Lung	0.162	2.08	2.21	32.4

## 2. Extraction effect of large and small molecule RNA from plant tissue samples

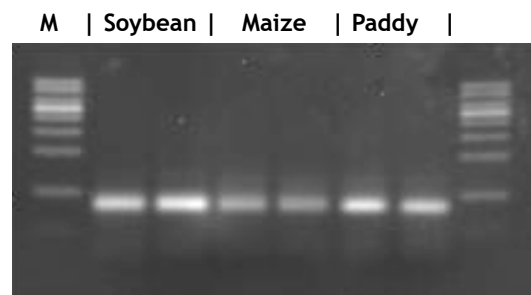
### 2.1 Extraction effect of small molecule RNA

Take 100mg soybean, maize, and paddy leaf samples, extract them according to the HiPure Universal miRNA Kit, measure the OD value of the extracted small molecule RNA using Nanodrop 2000, and analyze the band pattern using 3% agarose electrophoresis.

Sample	Conc. $\mu\text{g}/\mu\text{l}$	A260	A280	260/280	Yield $\mu\text{g}$
	0.268	6.699	4.054	1.65	16.1
Soybean	0.2364	5.91	3.457	1.71	14.2
	0.1776	4.44	3.148	1.41	10.7
Maize	0.1611	4.028	2.909	1.38	9.7
	0.0366	0.916	0.459	1.99	2.2
Paddy	0.0318	0.794	0.381	2.08	1.9

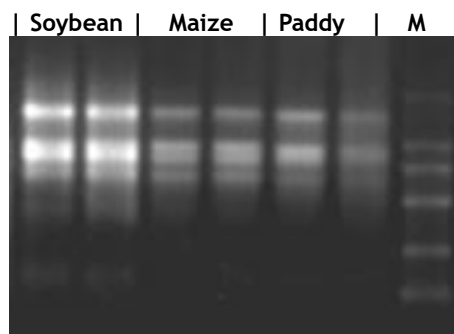
According to the data, OD260/280 is between 1.9~2.1, indicating high purity of small molecule RNA. According to the yield, the small molecule RNA content in 100mg soybean leaves is about 14-16 $\mu\text{g}$ .

Take ~1 $\mu\text{g}$  small molecule RNA and analyze by 3% agarose gel electrophoresis. The results are as follows. As shown in the figure, there is no contamination of large molecule RNA in the small molecule RNA obtained by this method. According to the DNA marker, small molecule RNA is mainly located in fragments below 50bp. This indicates that the kit can efficiently enrich small molecule RNA.



### 2.2 Extraction effect of large molecule RNA

The OD value of the extracted large molecule RNA was measured using Nanodrop 2000, and the band pattern was analyzed using 1.2% agarose electrophoresis. The results show that large molecular RNA contains almost no fragments below 200nt, indicating that small molecular RNA is not present. The OD reading indicates that the large molecular plant RNA obtained using HiPure Universal miRNA Kit has high purity and yield.



Sample	Conc. $\mu\text{g}/\mu\text{l}$	A260	A280	260/280	Yield $\mu\text{g}$
Soybean	0.3617	9.043	4.123	2.19	72.34
	0.4051	10.129	4.612	2.2	81.02
	0.062	1.55	0.752	2.06	12.4
Maize	0.0497	1.242	0.598	2.08	9.94
	0.0729	1.822	0.861	2.12	14.58
Paddy	0.0669	1.672	0.788	2.12	13.38