Carrier RNA R & D Report

Carrier RNA basic physical and chemical detection

1: Comparison of concentration and purity

- Weigh 20mg Carrier RNA dry powder by a 1mg level electronic balance, add 40ml RNase Free Water to dissolve, and make 500ng/μl.
- Take Carrier RNA products of B and C companies, add appropriate amount of RNase Free Water according to the instructions, and make 500ng/μl.

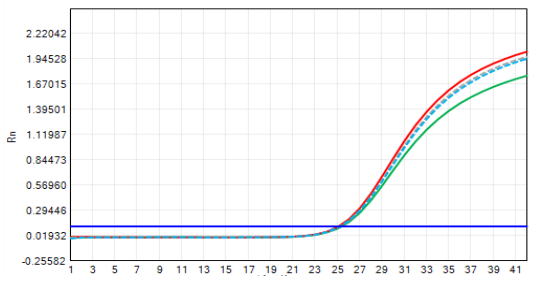
The concentration and purity of Carrier RNA was determined by Nanodrop 2100. The results are as follows.

Company	Weight Concentration	A260/230	A260/280	Result(ng/μl)
Magen	500ng/μl	4.62	2.96	530.90
A company	500ng/μl	4.43	2.92	566.95
B company	500ng/μl	4.78	3.01	473.52

Result analysis: Magen's Carrier RNA product OD value measurement concentration and weight concentration are comparable to Company A and Company B, indicating that the product has high purity and no other impurities in the Carrier RNA dry powder. Compared with import brands A and B, A260 / 230 and A260 / 280 are basically the same.

2. Influence on fluorescence quantitative RT-PCR

- Take the purified RNA product as a template for fluorescence quantitative RT-PCR, and add 0ug and 2μg of Mage Carrier RNA, and Carrier RNA of A and B companies to the mixture, and then perform quantitative RT-PCR to Check whether Carrier RNA inhibits quantitative RT-PCR results.
- Experimental results:

Dosage	Company	Ct	Amplification map
2μg	Magen	25.09	
2μg	PE	25.22	
2μg	Daan	25.19	
0μg		25.36	

Analysis of results: Fluorescence quantification results show that Carrier RNA does not inhibit fluorescence quantification, indicating that Magen's Carrier RNA is of high purity.

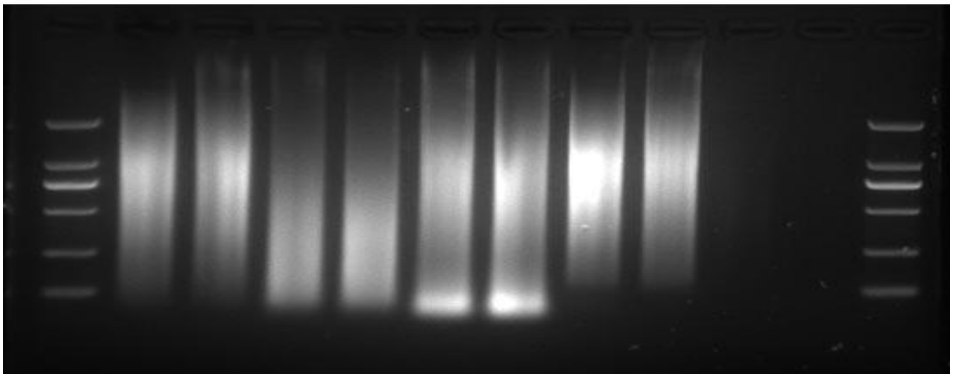
3. Fragment length analysis

- Take 2ug Carrier RNA (Magen, Company A and Company B), mix with 2μg Oligo (dT), and then use 1.5% agarose gel for electrophoresis analysis.

Experimental results:

Hybrid with Oligo dT

Marker | Magen | A company | B company | After purification | Carrier | dT | Marker



- Result analysis: electrophoresis results show that:

1: When pure Oligo dt and Carrier RNA are used for electrophoresis, there is no band. This is because single-stranded nucleic acid cannot bind to EB.

2: After hybridization with Oligo dT, Carrier RNA can be combined with EB, possessing electrophoresis conditions.

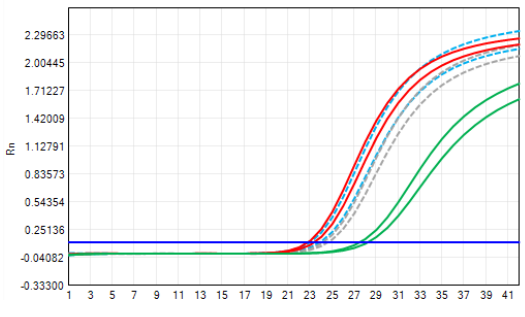
3: Compared with Company A and Company B, Magen Carrier RNA has a better polymerization effect, with the main fragments between 750-2000bp, while Company A has fragments between 100 ~ 500bp, Company B has small fragments, which may be insufficient polymerization products.

4: Magen Carrier RNA is purified by column method and purified under recovery conditions of removing fragments less than 200nt. The results show that the bands remained unchanged before and after purification, further indicate that there are very few short segments of Magen Carrier RNA.

二: Carrier RNA kit function development

4. Carrier RNA function development in the column virus extraction kit

- The influence of Carrier RNA of different company on the column method kit.

Sample	Carrier RNA	Company	Ct	Average	Amplification map
The Newcastle disease live vaccine (RNA virus) was diluted 1000-fold with sterile water and extracted with	0μg		27.49 28.24	27.87	
	4μg	Magen	22.77 23.33	23.05	
	4μg	A company	24.59 24.16	24.38	
	4μg	B company	23.01 23.89	23.45	

- Effect of Carrier RNA of Different Dosage on Column Kit R4173

Sample	Virus dilution factor	Dosage	Ct
The live Newcastle disease vaccine (RNA virus) was gradient diluted with pig plasma and extracted with Kit.	1000 times	0μg	25.52
		2μg	23.85
		4μg	23.50
		6μg	23.64
	10000 times	0μg	30.77
		2μg	27.46
		4μg	27.68
		6μg	27.08
	50000 times	0μg	NoCt
		2μg	32.06
		4μg	31.40
		6μg	31.00

5. Carrier RNA function development in magnetic bead virus extraction kit

- The effect of different amounts of Carrier RNA on the magnetic bead kit (IVD5412)

Sample	Virus dilution factor	Dosage	Ct
The live Newcastle disease vaccine (RNA virus) was gradient diluted with pig plasma and extracted with Kit.	1000 times	0μg	22.78
		3μg	22.72
		10μg	22.78
		20μg	22.65
	10000 times	0μg	26.54
		3μg	26.43
		10μg	26.37
		20μg	26.63
	50000 times	0μg	30.47
		3μg	30.54
		10μg	29.53
		20μg	30.29

Other R & D Data

1. R & D of Carrier RNA in column method and recovery rate of magnetic bead virus extraction kit.

- Take about 5ug Carrier RNA, dilute to 200μl with sterile water, add 200ul Buffer RL to vortex and mix well.
- Add 200μl of absolute ethanol for binding (the binding conditions can remove fragments below 200nt Poly A).
- Wash 600μl RW1 once and 600μl RW2 twice.
- 50μl Free Water for elution.

Carrier RNA column recovery test							
A260/230	A260/280	Result (ng/μl)	Condition	Yield	Company	Dosage	Recovery efficiency
0.50	3.05	113.68	200μl sterilized water + 200μlRL + 200μl ethanol	5.68	Magen	5.3μg	106%
0.47	3.02	114.08		5.70			
0.55	3.09	89.27		4.46	A company	5.6μg	71%
0.95	3.03	70.61		3.53			
0.21	3.04	59.50		2.97	B company	4.7μg	61%
0.18	3.06	56.89		2.84			

Carrier RNA recovery test in magnetic bead kit						
A260/230	A260/280	Result (ng/μl)	Yield	Company	Dosage	Recovery efficiency
0.11	2.68	69.37	3.47	Magen	5μg	70%
0.12	2.87	72.98	3.65			
0.10	2.54	62.09	3.10	A company	5μg	60%
0.09	2.64	58.67	2.93			
0.10	2.64	63.97	3.20	B company	5μg	60%
0.10	2.69	59.55	2.98			

Conclusion:

1. Using different magnetic bead methods to extract and recover a certain amount of carrier RNA, the recovery efficiency of Magen carrier RNA is basically between 60-70%, which is consistent with the carrier RNA recovery effect of Daan / PE Company, and even slightly better than other company's product.

2: Quantitative analysis of lyophilized powder

260/230	260/280	Dilute carrier RNA concentration 10 times (ng /μl)
4.05	2.87	112.57
4.36	2.93	108.83
4.33	2.93	123.71
4.31	2.89	115.23
4.44	2.99	107.92
4.57	2.98	122.38

Conclusion:

1. Take 6 tubes of 110ug lyophilized powder Carrier RNA, add 1.1ml of sterilized water to make it fully dissolved, and then measure the concentration. The results show that the concentration of the 6-tube lyophilized powder is not much different, indicating that the concentration of lyophilized powder is accurate.