Influence of Carrier RNA on A260/280 and A260/230

Carrier RNA, which is also known as poly A polyadenylation, is a mixture of oligomeric adenosine nucleotides with a length of 100~10000nt, formed by the polymerization of adenosine nucleotides by polynucleotide phosphorylase. In nucleic acid extraction, Carrier RNA is often added as an additive to lysis or binding solutions to improve the efficiency of nucleic acid extraction from trace samples. Due to the fact that Carrier RNA is an oligomer single stranded nucleotide, its OD260/280 and OD260/230 are relatively high, which is significantly different from double stranded DNA and total RNA (18S, 28S, mRNA).

Take 5mg Carrier RNA from different manufacturers, add 10ml DEPC treated water to dissolve it into 500g/μl, and then measure the OD value. The results are as follows. From the table, it can be seen that for purified Carrier RNA, OD260/280=2.9~3.0, A260/230= 4.0~5.0, while for double stranded DNA and total RNA (18S, 28S, mRNA), OD260/280=1.8~2.0, OD260/230= 1.5~2.5, which show significant difference.

Brand	Conc.	Conc. (ng/μl)	A260/280	A260/230
Magen	Take 5mg Carrier RNA dry powder and dissolve it into 500ng/μl	530.90	2.96	4.62
A Company		566.95	2.92	4.43
B Company		473.52	3.01	4.78

In Magen's products, D3125 (Trace DNA Extraction Kit), R4171 (Virus RNA Extraction Kit), R4173 (Virus DNA/RNA Extraction Kit), D3182 (Cell-free DNA Extraction Kit), IVD5412 (Magnetic Bead Virus RNA/DNA Kit), etc. all provide Carrier RNA to improve the nucleic acid recovery rate of trace samples. After extraction, Carrier RNA is also recovered and eluted into the product, with a recovery rate of up to 70~85%. When measuring with a spectrophotometer, the mixture of nucleic acid (DNA/RNA) and Carrier RNA can cause changes in OD260/280, OD260/230, and nucleic acid concentration. To verify the effect of Carrier RNA on OD values and concentrations in samples with different nucleic acid contents, we used ultrapure water (without nucleic acid), ultrapure water diluted DNA Marker (DNA), and bacterial culture media with different contents as samples, and then extracted nucleic acid using IVD541217 (magnetic bead method extraction kit), and measured OD values.

Sample	Kit	Carrier RNA	Conc. (ng/μl)	Yield (μg)	A260/A280	A260/A230
Ultrapure water	IVD541217	2.5 μg	26.87	2.42	3.45	3.12
			27.63	2.49	3.21	2.81
		0 μg	0.38	0.03	0.75	0.25
			0.35	0.03	0.53	0.26
Ultrapure water (Add 20μl DNA Marker)		2.5 μg	49.66	4.47	2.53	2.74
			51.30	4.62	2.50	2.70
		0 μg	20.51	1.85	1.84	2.10
			20.35	1.83	1.81	2.06
100μl LB Bacterial culture medium		2.5 μg	66.27	5.96	2.29	2.40
			67.00	6.03	2.29	2.40
		0 μg	51.90	4.67	2.11	2.14
			50.83	4.58	2.11	2.21
200μl LB Bacterial culture medium		2.5 μg	115.48	10.39	2.22	2.37
			117.64	10.59	2.24	2.39
		0 μg	103.45	9.31	2.13	2.26
			100.25	9.02	2.13	2.28

Conclusions:

1. When using ultrapure water without nucleic acid as the sample, Carrier RNA can be efficiently recovered by IVD541217, with a recovery rate of over 85%. At this point, OD260/280 and OD260/230 are essentially equivalent to oligonucleotides.

2. The effect of Carrier RNA on OD260/280 varies among samples with different concentrations. As the nucleic acid concentration increases, OD260/280 decreases from 3.4 to 2.2. This is because the proportion of Carrier RNA in the elution product gradually decreases, while double stranded DNA and total RNA in the cell increase, resulting in a more normal ratio. When the nucleic acid concentration rises to 60~70ng/μl and OD260/280<2.3, it has begun to approach the OD260/280=2.0~2.1 (total RNA). When it exceeds 60~70ng/μl, adding group A260/280 will increase by 0.1~0.2.

3. The effect of Carrier RNA on OD260/230 varies among samples with different concentrations. As the nucleic acid concentration increases, OD260/230 decreases from 3.1 to 2.3, which is also due to the gradual decrease in the proportion of Carrier RNA in the elution product, and the increase in cellular DNA and total RNA, resulting in a more normal ratio. When the nucleic acid concentration increased to 60~70ng/μl and OD260/230<2.4. Compared with the 100ng/μl experimental group, the difference in A260/230 ratio between the added group and the non-added group was 0.1~0.2, indicating that the higher proportion of double stranded DNA and total RNA, the less influence of carrier RNA.

4. In this experiment, only 2.5μg of Carrier RNA was added as an adjuvant to improve the nucleic acid recovery efficiency. However, in kits like R4171, R4173 (column method virus kits), the addition of Carrier RNA can reach up to 5μg, which has a more significant impact on nucleic acid concentration, OD260/230, and OD260/280. In addition, most of the biological samples processed by virus extraction kits are cell-free samples such as plasma, serum, culture medium supernatant, tissue homogenate supernatant, swab soaking solution, the nucleic acid content of which is quite low (<10ng/μl). After adding a large amount of Carrier RNA, the proportion of Carrier RNA in the elution product obtained is over 98~99%. Under these circumstances, the proportion and concentration obtained by OD measurement only reflect the properties of Carrier RNA and have little reference value.