Technical Data Sheet

FastGene® RNA Basic / Premium Kit DNase I Protocol Treatment

- Goal: Evaluation of the DNase I treatment after elution recommended in the FastGene® RNA Premium, and comparison to an on-column DNase I treatment.
- Materials:

FastGene® RNA Basic kit	6 Preps	Cat.No.FG-80006
	50 Preps	Cat.No.FG-80050
	250 Preps	Cat.No.FG-80250
FastGene® RNA Premium kit	6 Preps	Cat.No.FG-81006
	50 Preps	Cat.No.FG-81050
	250 Preps	Cat.No.FG-81250

Background

A DNase I treatment is not obligatory when using RNA purified with a silica membrane. However, for very DNA-sensitive downstream application, one of the following methods can be performed:

1. DNase I Treatment after elution: this is the standard protocol of the FastGene® RNA Premium kit
2. DNase I Treatment on column: optional protocol available

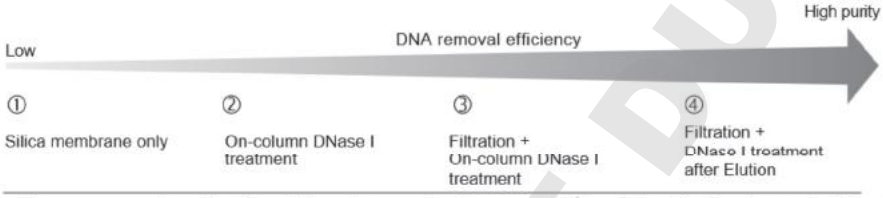
<DNase I treatment after elution>

In this method, impurities, such as salts, are removed during the RNA purifcation. This enables an enzymatic reaction in ideal conditions with high DNA removal efficiency.

<DNase I treatment on-column>

This method is widely used, due to its convenience. Here, the DNase I treatment is performed on column after binding the RNA to the column. This is, however, in a high-salt concentration environment, which affects the DNase I treatment efficiency. In order to avoid this, the column must be washed with adequate washing buffer. Failed removal of salts will result in a lower enzymatic activity and too low concentration of salts will cause the release of the RNA, resulting in reduced yields.

The following DNA removal efficiency is expected as follows:



Here, we present results of four different approaches to remove DNA and describe the observed efficiencies.

Experimental Conditions

Sample: Jurkat cell line
5× 10^5 cells /prep
n=3

Evaluated points:
1 Yield
2 RIN Score
3 Residual genomic DNA rate

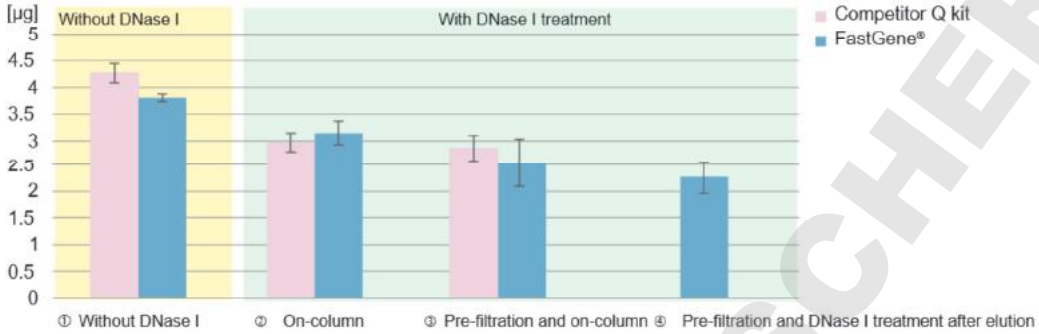
Test conditions

Kit name	DNase Treatment
FastGene® RNA Basic kit	① None
	② On -column
FastGene® RNA Premium kit	③ Filtration and on-column
	④ Filtration and after elution
Competitor Q	① None
	② On column
	③ Filtration and on-column

Results

Yield

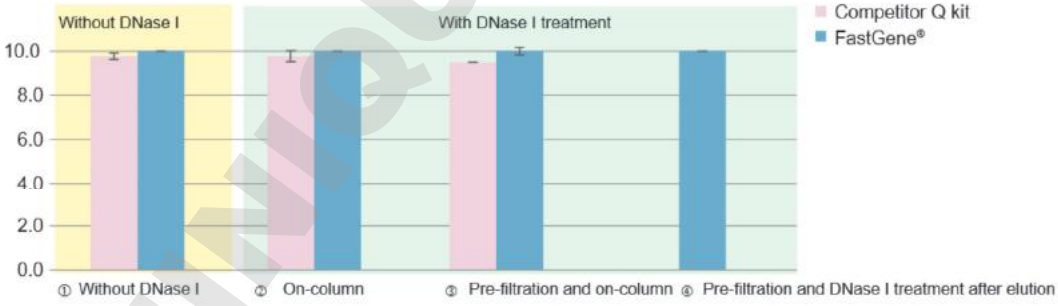
Product	DNase I Treatment	Results	
		Average Yield	Stand. Dev.
FastGene® RNA Basic kit	① None	3.81	0.07
	② On -column	3.15	0.22
FastGene® RNA Premium kit	③ Filtration and on-column	2.56	0.47
	④ Filtration and after elution	2.27	0.32
Competitor Q	① None	4.26	0.18
	② On column	2.97	0.18
	③ Filtration and on-column	2.85	0.24



All kits showed similar results under similar conditions.
Yields were in the following order:
No DNase I treatment > On-columen treatment > Filtration of sample before on-column treatment > Filtration before DNase I treatment after elution
Reason for this difference in the yield measurement could be the presence of residual genomic DNA.

RIN Score

Product	DNase I Treatment	Results	
		Average Yield	Stand. Dev.
FastGene® RNA Basic kit	① None	10	0
	② On -column	10	0
FastGene® RNA Premium kit	③ Filtration and on-column	10	0.2
	④ Filtration and after elution	10	0
Competitor Q	① None	9.8	0.2
	② On column	9.8	0.3
	③ Filtration and on-column	9.5	0



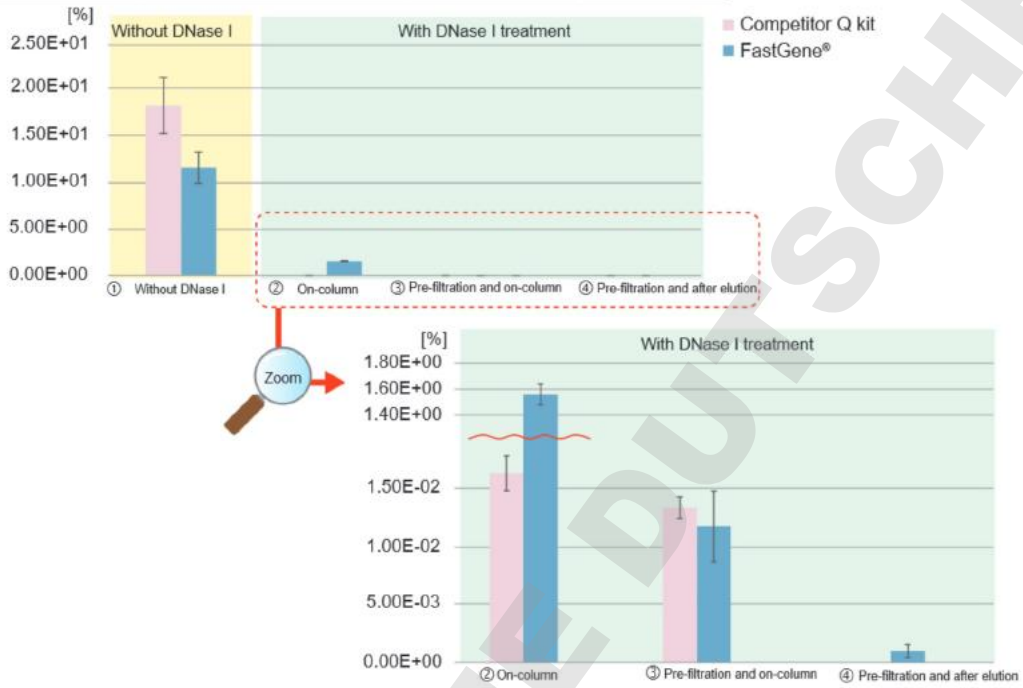
RIN score showed no difference under any condition.

Residual genomic DNA rate

Calculation of residual genomic DNA using absorbance and real-time PCR results:

$$\text{Residual genomic DNA\%} = \frac{\text{Amount of residual genomic DNA measured by qPCR [ng]}}{\text{Amount of RNA measured by absorbance [ng]}} \times 100$$

Product	DNase I Treatment	Results: Average Yield	Results: Stand. Dev.
FastGene® RNA Basic kit	① None	1.16× 10	1.65
	② On -column	1.56	8.07×10^{-2}
FastGene® RNA Premium kit	① Filtration and on-column	1.18×10^{-2}	3.00×10^{-3}
	④ Filtration and after elution	9.74×10^{-4}	5.41×10^{-4}
Competitor Q	① None	1.82× 10	2.98
	② On column	1.63×10^{-2}	1.47×10^{-3}
	③ Filtration and on-column	1.33×10^{-2}	9.13×10^{-4}



We can confirm the assumption stated by our company in the beginning: A DNase I treatment after elution showed the lowest amount of residual genomic DNA with a higher reproducibility, when compared to the other tested conditions

- Summary

Based on this experiment, the following results were obtained

- Yield:

①Without DNase I treatment >②On-column DNase I treatment >③ pre-filtration + on-column DNase I treatment >④pre-filtration + DNase I treatment after elution

- RIN Score:

The same tendency under any condition

- Genomic DNA removal efficiency:

①Without DNase I treatment <② On-column DNase I treatment <③ pre-filtration + on-column DNase I treatment <④pre-filtration + DNase I treatment after elution

Based on these results, the "DNase I treatment after elution" is recommended for the FastGene® RNA Premium Kit, for being the most effective for genomic DNA removal.

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