Performance of the RNA and the High Sensitivity RNA ScreenTape Assay for the Agilent 4200 TapeStation System

Technical Overview

Introduction

The Agilent 4200 TapeStation system provides an automated system for fast and reliable DNA and RNA electrophoresis with scalable throughput from 1 to 96 samples and full walk-away operation. The Agilent RNA ScreenTape assays have been developed for the separation and analysis of total RNA samples from eukaryotic or prokaryotic origin providing qualitative and quantitative information. The RNA integrity number equivalent (RIN^e) delivers an objective assessment of eukaryotic and prokaryotic total RNA degradation[1]. The 4200 TapeStation system and the RNA ScreenTape assays are ideally suited for RNA quality control within RNAseq, microarray analysis, and quantitative RT-PCR workflows.

This Technical Overview focuses on the performance of the 4200 TapeStation with both RNA ScreenTape assays. Quantification data and RIN^e values were directly correlated to the Agilent 2200 TapeStation system to demonstrate the full compatibility of both systems. Table 1 summarizes the analytical specifications of the RNA ScreenTape assays.

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Table 1. Analytical specifications of the Agilent RNA and the Agilent High Sensitivity RNA ScreenTape assays for the Agilent 4200 TapeStation system.

Analytical specifications	Agilent RNA ScreenTape Assay	Agilent High Sensitivity RNA ScreenTape Assay
Quality score	RIN^e	RIN^e
RIN^e functional range	25–500 ng/µL	1,000–25,000 pg/µL
Analysis type	Eukaryotic or prokaryotic total RNA QC	Eukaryotic or prokaryotic total RNA QC
Sensitivity[1]	5 ng/µL	100 pg/µL
Quantitative range	25–500 ng/µL	500–10,000 pg/µL
Quantitative precision	10 %CV	15 %CV
Quantitative accuracy[2]	± 20 %	± 30 %
Maximum sample buffer strength	200 mM Tris, 20 mM EDTA, or 50 mM NaCl	10 mM Tris, 1 mM EDTA

[1] Signal-to-noise (S/N) > 3 (single peak)
[2] Measured against an Agilent 2200 TapeStation system

Experimental

Materials

The Total RNA, Kidney, Sprague-Dawley Rat, Male (p/n 737007), the Agilent 4200 TapeStation (p/n G2991AA), and Agilent 2200 TapeStation (p/n G2964AA or G2965AA) system with Agilent RNA ScreenTape (p/n 5067-5576), Agilent RNA ScreenTape Sample Buffer (p/n 5067-5577), Agilent RNA ScreenTape Ladder (p/n 5067-5578), Agilent High Sensitivity RNA ScreenTape (p/n 5067-5579), Agilent High Sensitivity RNA ScreenTape Sample Buffer (p/n 5067-5580), and Agilent High Sensitivity RNA ScreenTape Ladder (p/n 5067-5581) were obtained from Agilent Technologies (Waldbronn, Germany). The ND2000 NanoDrop UV/Vis spectrophotometer was purchased from Thermo Fischer Scientific Inc. (Wilmington, DE, USA).

Sample preparation

The male rat total RNA was incubated at 95 °C over a time course to yield four samples with different degrees of RNA integrity. The RNA concentration was determined using UV-Vis spectrophotometry. Based on the quantification, the samples were diluted to the described nominal concentration in water.

RNA analysis

The RNA analysis was performed using the RNA or the High Sensitivity RNA ScreenTape assays and both the 2200 and 4200 TapeStation systems according to the manufacturer[2-5]. On both platforms, TapeStation software revisions A.02.01 were used for data analysis.

Results and Discussion

Total RNA Quality Analysis

Four different male rat RNA samples with varying RNA degradation were analyzed using the 4200 TapeStation system. The 4200 TapeStation Software displays the results as an electropherogram, a gel image, and in a data table. The RIN^e value is automatically determined and directly displayed under the individual lane of the gel image (Figure 1). RIN^e is calculated at a scale from 1 to 10. A high RIN^e indicates highly intact RNA, and a low RIN^e a strongly degraded RNA sample.

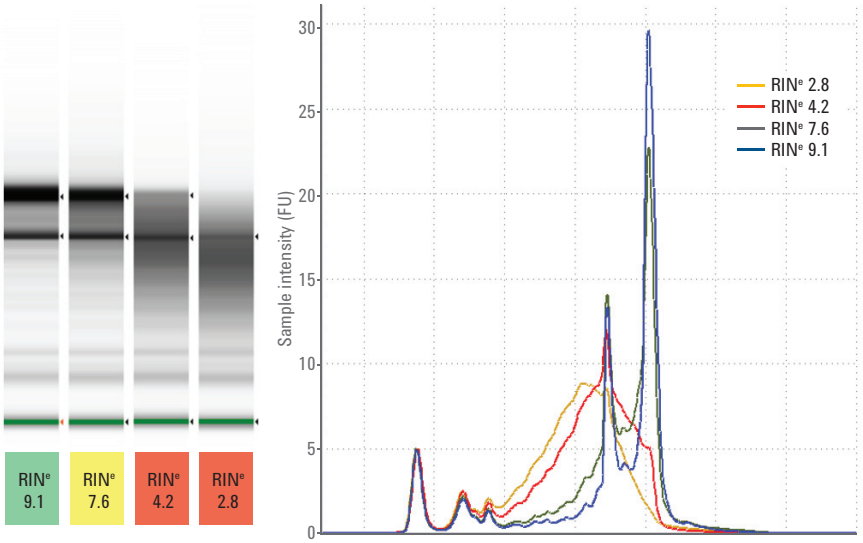

Figure 1. Four rat total RNA samples (300 ng/µL) with different levels of RNA degradation were analyzed using the Agilent 4200 TapeStation system and the Agilent RNA ScreenTape assay. The determined RIN^e values are shown under the gel image.

For a direct correlation of the obtained RIN^e values, the same total RNA samples were analyzed across two different ScreenTape devices on the 4200 TapeStation and the 2200 TapeStation system (n = 8 per sample per instrument).

Figure 2 summarizes the data obtained for the RNA quality analysis using the 4200 TapeStation and the 2200 TapeStation systems. The RIN^e obtained with both TapeStation systems can be considered equivalent, with an $R^2 = 0.9992$ for the RNA ScreenTape assay, and an $R^2 = 0.9996$ for the High Sensitivity RNA ScreenTape assay.

In addition, RIN^e determination is highly reproducible. For both TapeStation systems and both RNA assays, reproducibility is typically below 5 % CV (data not shown).

To demonstrate the stability of the RIN^e determination across a full plate, four RNA samples with different degrees of degradation were analyzed with the RNA ScreenTape assay on the 4200 and the 2200 TapeStation systems. The data demonstrate that the determination of RIN^e is very reproducible and stable across a full plate (Figure 3). No RNA degradation is observed during the analysis using the RNA ScreenTape assay and the High Sensitivity RNA ScreenTape assay (data not shown).

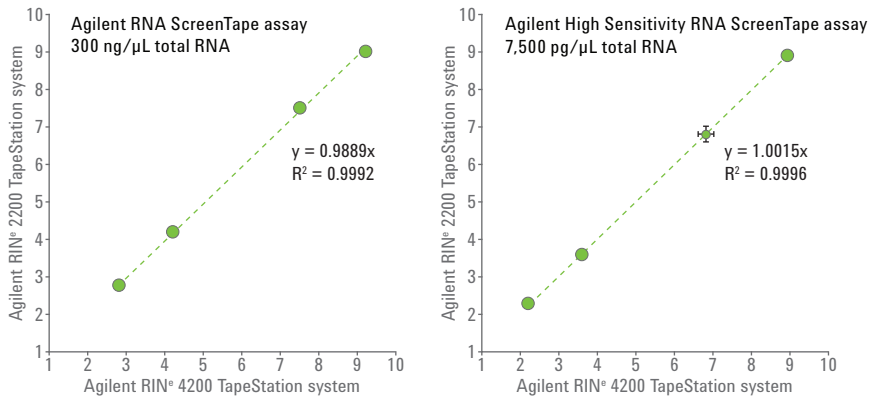

Figure 2. Total RNA samples with different degrees of RNA degradation were analyzed using the Agilent 4200 TapeStation and the Agilent 2200 TapeStation system, and both Agilent RNA ScreenTape assays. The averaged RIN^e and standard deviations are plotted in this graph (n = 8 for each sample).

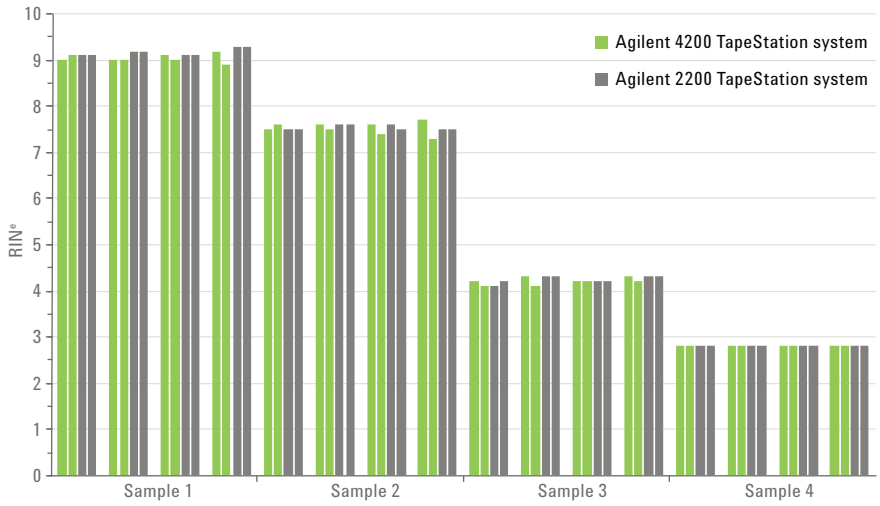

Figure 3. Total RNA samples with different degrees of RNA degradation were analyzed using the Agilent 4200 TapeStation and the Agilent 2200 TapeStation system, and the Agilent RNA ScreenTape assay.

Sensitivity

Intact rat total RNA at a concentration of 5 ng/µL was analyzed across a 96-well plate with the RNA ScreenTape assay, and at 100 pg/µL with the High Sensitivity RNA ScreenTape assay using the 4200 TapeStation system. Figure 4 shows the electropherogram overlay of multiple runs.

The signal peaks from 5 ng/µL total RNA analyzed with the RNA ScreenTape assay, and from 100 pg/µL analyzed with the High Sensitivity RNA ScreenTape assay are clearly visible above the baseline, with an S/N greater than 3. This confirms the specified sensitivity for both RNA ScreenTape assays (Table 1).

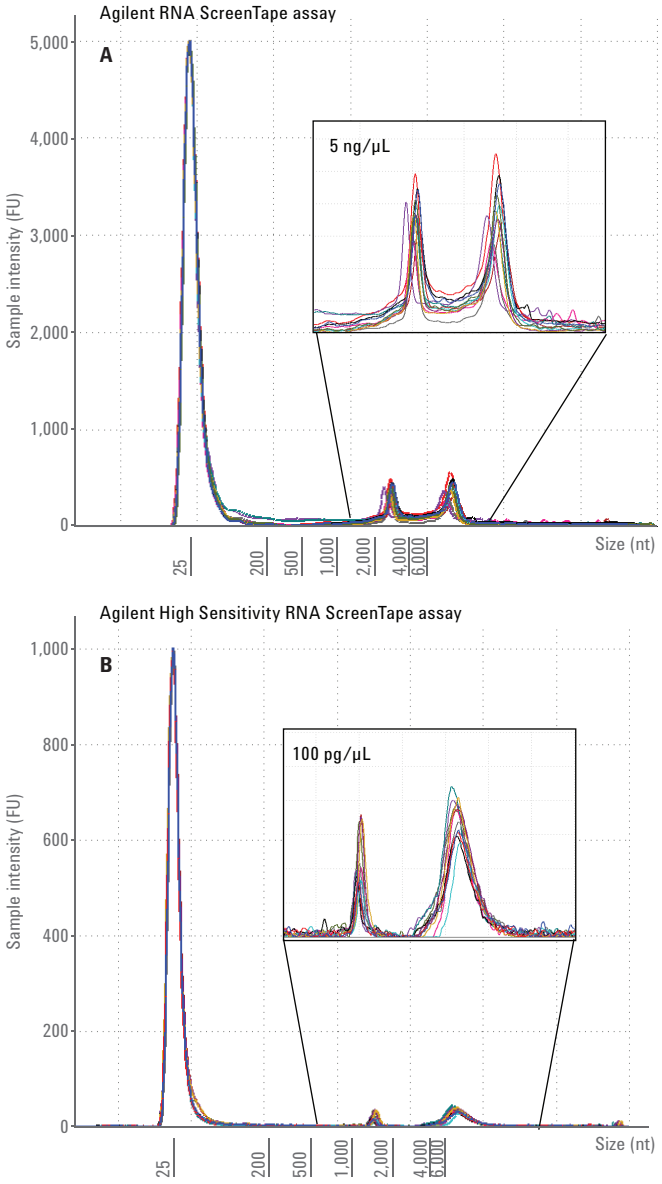

Figure 4. A) Electropherogram overlay shows 12 replicate runs of 5 ng/µL RNA with the Agilent RNA ScreenTape assay, and B) 100 pg/µL RNA with the Agilent High Sensitivity RNA ScreenTape assay using an Agilent 4200 TapeStation system. The inserts shows an enlargement of the electropherograms.

RNA Quantification

To demonstrate the quantification precision of the RNA ScreenTape assays on the 4200 TapeStation system, intact rat total RNA at different concentrations were run across a 96-well plate on the 4200 TapeStation system and with 8-way tube strips using the 2200 TapeStation system. Figure 5 summarizes the quantification data obtained for both the RNA and the High Sensitivity RNA ScreenTape assay.

The average quantitative precision for the 4200 TapeStation system was well within the specifications for the RNA ScreenTape assay and the High Sensitivity RNA ScreenTape assay (Table 1).

Conclusion

This Technical Overview shows that the Agilent 4200 TapeStation system with the Agilent RNA and the Agilent High Sensitivity RNA ScreenTape assays provides efficient and reliable RNA analysis, including RNA characterization and quality assessment. RIN^e allows an accurate and objective assessment of total RNA degradation of eukaryotic or prokaryotic samples. The RIN^e as well as the quantification and sizing data obtained with the 4200 TapeStation system is highly equivalent to the data obtained using the Agilent 2200 TapeStation system. In comparison to the 2200 TapeStation system, the 4200 platform offers an increased walk away time, as the system can analyze up to 96 samples in an unattended way.

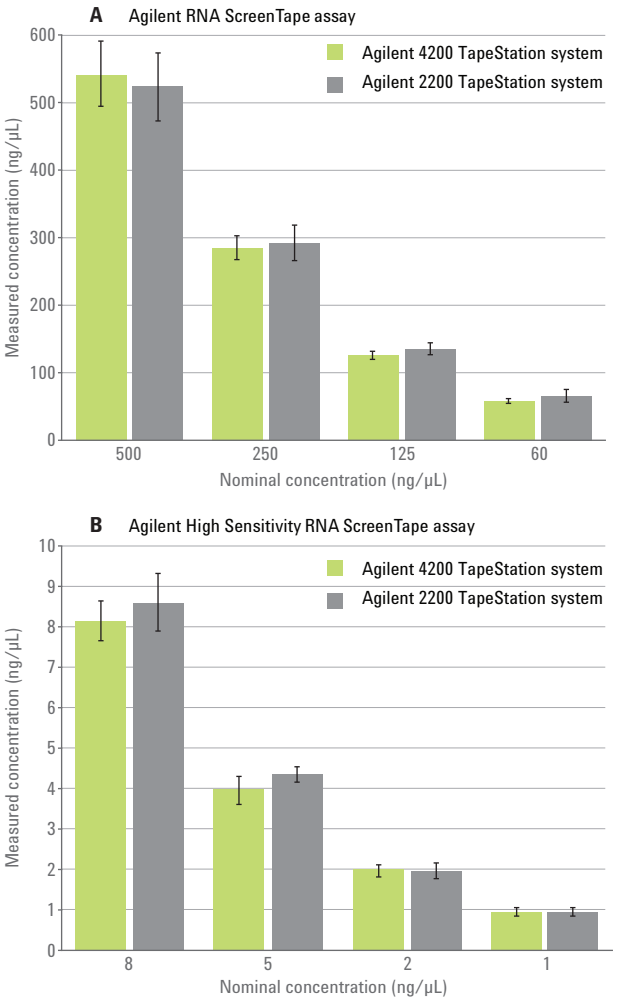

Figure 7. Intact rat total RNA samples with nominal concentrations ranging from 60 to 500 ng/µL were quantified using the Agilent RNA ScreenTape assay and with concentrations from 1 to 8 ng/µL with the Agilent High Sensitivity ScreenTape assay using an Agilent 4200 TapeStation (n = 24) and an Agilent 2200 TapeStation system (n = 12). The error bar indicates the standard deviation.

References

1. RNA quality control using the Agilent 2200 TapeStation system – Assessment of the RIN^e quality metric, *Agilent Technologies*, publication number 5991-0023EN, **2012**.

2. Agilent RNA ScreenTape System Quick Guide, *Agilent Technologies*, publication number G2964-90022 Rev. D, **2014.**

3. Agilent High Sensitivity RNA ScreenTape System Quick Guide, *Agilent Technologies*, publication number G2964-90121 Rev. D, **2014.**

4. Agilent RNA ScreenTape Assay Quick Guide for 4200 TapeStation System, *Agilent Technologies*, publication number G2991-90020 Rev. B, **2015.**

5. Agilent High Sensitivity RNA ScreenTape Assay Quick Guide for 4200 TapeStation System, *Agilent Technologies*, publication number G2991-90120 Rev. B, **2015.**

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