Standard Sensitivity RNA Assay _ TapeStation 4200 Quick Guide

The Standard Sensitivity RNA ScreenTape system is designed for analyzing eukaryote and prokaryote RNA from 50 to 6000 nucleotides. Contaminating intact genomic DNA (20-40kb gDNA) is not detected.

This kit is used for total RNA ranging from 5 ng/µl to 500 ng/µl, with an ideal range between 25 and 500 ng/µl.

The TapeStation 4200 (TS4200) separates RNA by electrophoresis in a chip called ScreenTape. Each ScreenTape has 16 lanes, which are single-use hence completely eliminating contamination and carryover. In a single run, 1 to 96 samples can be analyzed.

The run being very fast, no booking is needed.

Labcoats and goggles are available near the TS4200 or just outside the main room. Inquire if needed.

30 minutes before starting instrument/samples preparation (=50 min before the actual run)

Take out of the *fridge* and put at room temperature (for approximately 30 minutes):

RNA Sample buffer (*not high sensitivity…)*	Stored in the fridge in the TS4200 box. If multiple tubes are present, use the one marked with a dot (If no dot is present, draw it on the tube you use).

Take out of the *-20° freezer* and keep on ice:

RNA ladder	Stored in -20° freezer, drawer “FA and TS4200”, in the TS4200 box.

Take out of the *-80° freezer* and keep on ice:

Standard Sensitivity RNA positive control aliquot	Stored in the box called “FA positive control”. Check the RNA concentration written on the bag.

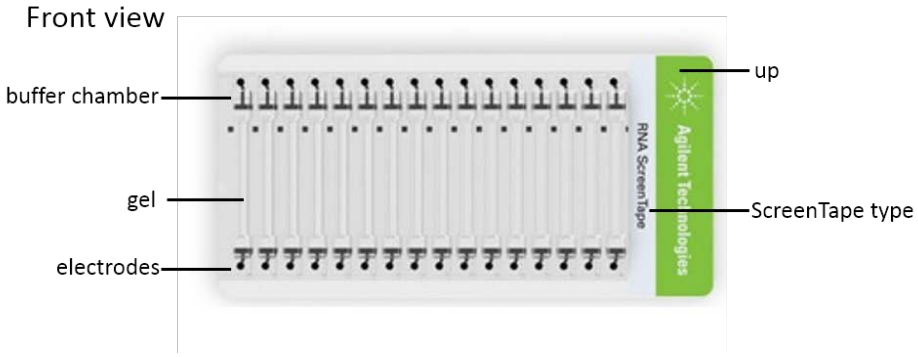
Write down on the log sheet (attached to the fridge next to the TS4200) your name, PI and **number of samples** (do not include ladder and positive control). Billing is done according to this information.

Instrument preparation (5 minutes required)

1. Switch on the instrument.
2. Switch on the TS4200 Controller Software.
3. Determine how many lanes you need according to your number of samples + positive control + ladder. The ladder is loaded only once even if you use multiple ScreenTapes.
4. Determine whether partially-used RNA ScreenTapes (green and NOT labelled “high sensitivity”) are available on the white rack in the fridge. These **must** be used in priority. *Note: you can still use an open ScreenTape even if there is only one lane left.*

Note: How to know how many valid lanes are left in an open ScreenTape and whether it is expired?

- *Check what it is written on the envelope (date of opening & number of lanes used). ScreenTapes expire 2 weeks after opening.*
- *Look vertically at the buffer chamber, used ones have a hole on the top.*
- *If still unclear, insert the ScreenTape in the TS4200, the instrument displays its expiration date on the bottom left of the software window. The used wells appear in dark grey on the scheme.*



5. Take out of the fridge partially-used or new RNA ScreenTapes as needed.
6. Holding the ScreenTape with the front side facing you, tap it few times (to bring on top of the buffer chamber putative air bubbles)
7. With the ScreenTape front side facing you, insert it in the ScreenTape nest. Make sure the QR reader reads the QR code located on the backside. If you have multiple ScreenTapes, load the partially used one in the loading nest, and the new ones in the ScreenTape rack (on the left). Load them starting from the most rear position, with their front side facing you.
8. Determine if you will use strips or a 96-well plate, according to these guidelines:
    a. If you need 16 lanes or less in total, you can use either 8-well strips or 96-well plates. *Note: If you use 2 or more ScreenTapes with strips, you'll have to stay around to add the new ScreenTape manually when prompted during the run.*
    b. If you need more than 16 lanes in total, you have to us a 96-well plates.
9. Write the name of your samples in the software, by selecting the wells to be used, according to these **IMPORTANT NOTES**:
    a. Ladder always go to position **A1 of an 8-well strip**, even if you use a 96-well plate.
    b. Load your samples in a **vertical order** (A1, B1, C1…).
    c. Indicate in the name the information of putative samples dilutions.
10. The instrument calculates how many tips and ScreenTape are required and shows them in the "Required for the run" window.
11. Check that you have loaded the correct number of ScreenTapes.
12. Check if enough tips are present, and if needed add tips or load a new tips rack.
13. Close the instrument lid.

Sample preparation (10-15 minutes required)

14. Get the required material for sample preparation (8-well strip and caps, or 96-well plate and foil seal) from the boxes that are next to the instrument or on top of the fridge.
15. Pre-heat a Sensoquest thermocycler at 72°C ("run"-> Denaturation -> 72°C).

16. Vortex all reagents prior to use.
17. In position A1 of an 8-well strip, mix 5 µl of Sample buffer (green lid) and 1 µl of ladder (yellow lid). Mark the position on the tube to know where the ladder is loaded.
18. For each sample, pipette 5 µL of Sample Buffer (green lid) and 1 µL of RNA sample in a well plate or tube strip.
19. Apply foil seal to well plate and caps to tube strips.
20. Mix samples/ladder plates/strips using the IKA vortex at 2000 rpm for 1 min (just press start).
21. Spin down the plate/strip in one of our plate/strip tabletop centrifuges.
22. Heat samples, positive control, **and ladder** to 72°C for 3 minutes, by inserting the samples in the thermocycler and selecting Run ->Continue to move the lid down and start the countdown.
23. Place samples and ladder on ice for 2 min.
24. Spin down to position the samples and ladder at the bottom of the plate/ strip and make sure that there are **no bubbles left**.

Start the run

25. Carefully **remove caps of tube strips**. Don't remove the foil seal from the 96-well plate.
26. Remove the **lid from the tips rack**.
27. Load samples and ladder plate/strip into the instrument.
28. Make sure ladder is in position A1 on tube strip holder.
29. Press Start. The software then soon displays the run time (2-3 minutes per sample).
30. If running several ScreenTapes with strips, you have to stay around and manually change ScreenTape when prompted.
31. Place reagents back to their respective storage places.

End of the run

32. Remove everything from the instrument. Empty the bins and throw away tube strip or plates.
33. Replace the lid on the tips rack.
34. Indicate on the ScreenTape envelope the number of used lanes. If a new ScreenTape was opened, indicate the date.
35. If there are unused lanes left on the ScreenTape, place it **upright** in the white rack at 4°.
36. Close the TS4200 Controller Software. Let the PC switched on.
37. Switch off the instrument.
38. Log your run into the logging/billing sheet. Report if reagents should be reordered.

Data Analysis

39. The TS4200 Analysis Software opens after the run and displays results.
40. Clicking on the “gel” and “electropherogram” buttons displays the gel image and the profile.
41. On the bottom part there is a table reporting concentration and RIN information.
42. Click on “File/Create Report” to save a PDF file report that contains gel image, profile, concentration and RIN information. Collect both the pdf and the TS4200 file for your records.

Troubleshooting

43. By unselecting the “Aligned” button, the software displays the raw data, prior to alignment of the lower marker and normalization (useful for troubleshooting).
44. By selecting the “Scale to sample” button, the software displays a gel image that it is scaled for each sample individually (useful when samples have very different concentrations).

Versions log

- v1.0: Initial release.

- v1.1: Multiple minor changes.

- v1.2: Edited the guidelines to account for new possibility to run multiple ScreenTapes from strips. Added information regarding labcoats and goggles.