Agilent DNF-472 HS RNA (15 nt) Kit Quick Guide for the Fragment Analyzer Systems

The Agilent Fragment Analyzer systems are automated capillary electrophoresis platforms for scalable, flexible, fast, and reliable electrophoresis of nucleic acids.

This Quick Guide is intended for use with the Agilent 5200, 5300, and 5400 Fragment Analyzer systems only. This kit is designed to analyze Total RNA at a concentration of 50 pg/µL – 5,000 pg/µL input sample concentration and mRNA at an input sample concentration of 500 pg/µL – 5,000 pg/µL .

Specifications

Analytical specifications[1,2]	HS RNA assay (15nt)	
Sizing Range	200 nt – 6,000 nt	
Sizing Accuracy[1]	± 20%	
Sizing Precision[1]	20% CV	
Limit of Detection (S/N > 3)	50 pg/µL – Total RNA	250 pg/µL –mRNA
Quantitative Range (per smear)	50 pg/µL – 5,000 pg/µL – Total RNA	500 pg/µL – 5,000 pg/µL mRNA
Quantification Accuracy[1]	± 30%	
Quantification Precision[1]	20% CV	
Physical Specifications[3]		
Total electrophoresis run time	22cm: 31 minutes, 33cm: 40 minutes, 55cm: 70 minutes	
Samples per run	12, 48 or 96; depending on the instrument type	
Sample volume required	2 µL	
Kit stability	4 months	

1 Results using RNA Ladder as sample and 33-55 capillary array.
2 Results using Total RNA and ribo-depleted mRNA samples diluted in nuclease-free water.

Kit Components – 500 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6574*		HS RNA (15 nt), 500, 4°C	
	DNF-265-0240	RNA Separation Gel, 240 mL	1
	DNF-301-0008	BF-1 Blank Solution, 8mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL • Dilute with sub-micron filtered water prior to use	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-472-FR*		HS RNA (15 nt) FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-370-0004	HS RNA Diluent Marker (15 nt), 4 mL	3
	DNF-386-U015	HS RNA Ladder, 15 µL	1
5191-6612*		Quantitative DNA, RT	
	C275-130	Eppendorf LoBind 0.5 mL tubes (bag of 50)	1
	DNF-475-0050	5x Capillary Conditioning Soln, RT • Dilute with sub-micron filtered water prior to use	1

*Not orderable.

WARNING

- Refer to product safety data sheets for further information
- When working with the Fragment Analyzer kit components follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Kit Components – 1000 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6575*		HS RNA (15 nt), 1000, 4°C	
	DNF-265-0500	RNA Separation Gel, 500 mL	1
	DNF-301-0008	BF-1 Blank Solution, 8mL	1
	DNF-355-0300	5x 930 dsDNA Inlet Buffer, 300 mL • Dilute with sub-micron filtered water prior to use	1
	DNF-497-0250	0.25x TE Rinse Buffer, 250 mL	1
DNF-472-FR*		HS RNA (15 nt), FR	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	DNF-370-0004	HS RNA Diluent Marker (15 nt), 4 mL	6
	DNF-386-U015	HS RNA Ladder, 15 µL	2
5191-6613*		HS RNA (15 nt), 1000, RT	
	C275-130	Eppendorf LoBind 0.5 mL tubes (bag of 50)	1
	DNF-475-0100	5x Capillary Conditioning Soln, RT • Dilute with sub-micron filtered water prior to use	1

*Not orderable.

WARNING

- Refer to product safety data sheets for further information
- When working with the Fragment Analyzer kit components follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

NOTE: RNA samples and RNA Ladders are very sensitive to RNase contamination, which can lead to experimental failure. To minimize RNase contamination, wear gloves when working with RNA samples and reagents, and when handling accessories that will come in contact with the RNA sample. Use certified RNase-free plastics and disposable consumables. It is also recommended to work in a separate lab space if possible and decontaminate the pipettes and work surface to avoid cross contamination.

Additional Material Required for Analysis with the Fragment Analyzer Systems

- Fragment Analyzer systems with LED fluorescence detection:
- 5200 Fragment Analyzer system (p/n M5310AA)
    - FA 12-Capillary Array Ultrashort, 22 cm (p/n A2300-1250-2247) OR
    - FA 12-Capillary Array Short, 33 cm (p/n A2300-1250-3355) OR
    - FA 12-Capillary Array Long, 55 cm (p/n A2300-1250-5580)
- 5300 Fragment Analyzer system (p/n M5311AA)
    - FA 48-Capillary Array Short, 33 cm (p/n A2300-4850-3355) OR
    - FA/ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
    - FA/ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580)
- 5400 Fragment Analyzer system (p/n M5312AA)
    - FA 48-Capillary Array Short, 33 cm (p/n A2300-4850-3355) OR
    - FA/ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
    - FA/ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580):
- Agilent Fragment Analyzer controller software (Version 1.1.0.11 or higher)
- Agilent ProSize data analysis software (Version 2.0.0.61 or higher)

Additional equipment/reagents required (not supplied)

- 96-well PCR sample plates. Please refer to Appendix – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer System User Manual for a complete approved sample plate list
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 µL volumes (sample plates) and 1,000 µL volumes (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 5x 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solution)
- 96-deepwell 1mL plate: Fisher Scientific #12-566-120 (inlet buffer and/or waste plate)
- Reagent reservoir, 50 mL (VWR #89094-680 or similar) (for use in pipetting inlet buffer plates/sample trays)
- Conical centrifuge tubes for prepared separation gel/dye mixture and/or 1x Capillary Conditioning Solution
    - 50 mL (for 5200 Fragment Analyzer system or 50 mL volumes): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
- 250 mL (for 5300 and 5400 Fragment Analyzer systems or larger volumes): Corning #430776, available from Fisher Scientific #05-538-53 or VWR #21008-771
- Vortexer (for mixing of samples, ladders, and/or markers in tubes and/or plates)
- Capillary Storage Solution (p/n GP-440-0100)

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
Steps before sample preparation	• Allow reagents to equilibrate at room temperature for 30 min prior to use
Pipetting practice	• Pipette reagents carefully against the side of the 96-well sample plate or sample tube • Ensure that no sample or Diluent Marker remains within or on the outside of the tip

HS RNA Ladder Preparation

Upon arrival of the ladder, it is recommended to divide the ladder into 3 µL aliquots. Store aliquots in the provided Eppendorf LoBind 0.5 mL tubes at -70°C or below.

1. Thaw a 3 µL 25 ng/µL ladder aliquot on ice.

2. Spin down the contents and mix by pipetting the solution up and down with a pipette tip set to a 2 µL volume.

   a) Transfer 2 µL of the 25 ng/µL Ladder to a fresh Eppendorf LoBind 0.5 mL tube.

   b) If more than 2 µL of the 25 ng/µL is transferred for heat-denaturing, be sure to add enough RNase-free water to dilute the ladder to the working concentration of 2 ng/µL.

   c) Heat-denature the ladder at 70°C for 2 min, immediately cool to 4°C and keep on ice.

3. Dilute the ladder solution to a working concentration of 2 ng/µL by adding 23 µL of RNase-free water and mixing well. Divide the diluted ladder solution into aliquots with working volume typical for one day use or one sample plate. Store aliquots in the provided Eppendorf LoBind® 0.5 mL tubes at -70°C or below.

Total RNA Sample Preparation

1. Heat-denature the RNA samples at 70°C for 2 min if needed and immediately cool to 4°C and keep on ice before use.

2. The above RNA sample concentrations assume the sample is in water. If salt is present, some loss of sensitivity may be observed and slight adjustments may need to be made to the sample injection conditions.

mRNA Sample Preparation

1. Heat-denature the RNA samples at 70°C for 2 min if needed and immediately cool to 4°C and keep on ice before use.

2. The mRNA input sample must be within a total concentration range of 500 pg/µL to 5000 pg/µL for optimal assay results. If the concentration of the sample is above this range, dilute with RNase-free water.

Sample Plate Preparation

1. The total input RNA sample concentration MUST be within a range of 50 pg/µL to 5000 pg/µL (total RNA) or 500 pg/µL to 5000 pg/µL (mRNA) for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with RNase-free water prior to performing the assay.

2. The above RNA sample concentrations assume the sample is in water. If salt is present, some loss of sensitivity may be observed and slight adjustments may need to be made to the sample injection conditions.

3. Using a fresh RNase-free 96-well sample plate, pipette 18 µL of the HS RNA Diluent Marker (15 nt) (DM) Solution to each well in a row that is to contain sample or RNA Ladder. Fill any unused wells within the row of the sample plate with 20 µL/well of BF-1 Blank Solution.

4. Pipette 2 µL of each denatured RNA sample into the respective wells of the sample; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

5. RNA Ladder: The RNA Ladder must be run in parallel with the samples for each experiment to ensure the accurate quantification. Thaw the denatured 2 ng/µL working concentration RNA Ladder on ice. Pipette 2 µL of denatured RNA Ladder into the 18 µL of Diluent Marker (15 nt) (DM) Solution in the designated ladder well:

- 12-Capillary System: Well 12 of each row to be analyzed
- 48-Capillary System: Well D12 or H12, depending on which group is chosen.
- 96-Capillary System: Well H12

6. Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip or use one of the mixing methods suggested in the following.

7. After mixing sample/RNA Ladder and Diluent Marker (15 nt) Solution in each well, centrifuge the plate to remove any air bubbles. Check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. The presence of trapped air bubbles can lead to injection failures.

8. For best results, run the plate as soon as possible. If the sample plate will not be used immediately, cover the sample plate with RNase-free cover film, store at 4°C and use within the same day. Spin the plate again if any bubbles developed in the sample wells. Be sure to remove the cover film before placing the plate into the instrument.

9. To run the samples:

- In the 12-Capillary System, place the plate in one of the three sample plate trays (Drawers 4-6 from the top).
- In the 48-Capillary System, place the plate in one of the three sample plate trays (Drawers 4-6 from the top).
- In the 96-Capillary System, place the plate in one of the two available sample plate trays (Drawers 4-5 from the top).

10. Load or create the experimental method as described in the following sections.

Important Sample Mixing Information

When mixing sample with diluent marker solution, it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. It is highly suggested to perform one of the following methods to ensure complete mixing:

- When adding 2 µL of sample or ladder to the 18 µL of diluent marker, swirl the pipette tip while pipetting up/down to further mix.
- After adding 2 µL of sample or ladder to the 18 µL of diluent marker, place a plate seal on the sample plate and vortex the sample plate at 3000 rpm for 2 min. Any suitable benchtop plate vortexer can be used. Ensure that there is no well-to-well transfer of samples when vortexing. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells.
- After adding 2 µL of sample or ladder to the 18 µL of diluent marker, use a separate pipette tip set to a larger 20 µL volume, and pipette each well up/down to further mix.
- Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample volume. Some models enable using the pipette tip for both adding and mixing.

NOTE: Avoid total input RNA sample concentrations above the specified limits. Overloading of RNA sample can result in saturation of the CCD detector and poor results. The peak heights for RNA smears should lie in an optimal range between 20 – 2000 RFUs. The peak heights for individual RNA fragments in total RNA should lie in an optimal range between 100 – 20,000 RFUs.

Gel preparation

Prepare gel/dye mixture for 5200, 5300, and 5400 Fragment Analyzer Systems. To ensure the gel/dye mixture is mixed homogeneously without generating bubbles, gently invert the centrifuge tube 5 to 10 times, depending on the volume of the mixture. NOTE: Centrifuge dye prior to opening the vial to reduce risk of leaking.

5200 Fragment Analyzer system volume specifications

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
12	1.0 µL	10 mL	10 mL
24	1.5 µL	15 mL	15 mL
36	2.0 µL	20 mL	20 mL
48	2.5 µL	25 mL	25 mL
96	4.5 µL	45 mL	45 mL

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

5300 Fragment Analyzer system volume specifications with 48-capillary array

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
48	2.5 µL	25 mL	25 mL
96	4.0 µL	40 mL	40 mL
144	5.5 µL	55 mL	55 mL
192	7.0 µL	70 mL	70 mL
240	8.5 µL	85 mL	85 mL
288	10.0 µL	100 mL	100 mL

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

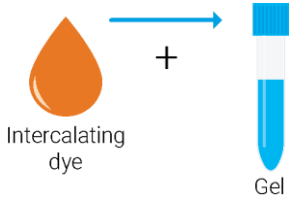
5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

Agilent HS RNA (15nt) DNF-472 assay operating procedure

1. Mix fresh gel and dye according to the volumes in the Gel preparation tables. Refill 1x Capillary Conditioning Solution as needed.



2. Place a fresh 1x 930 dsDNA Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.

   2.1. 5200 system; Fill row A of buffer plate

   2.2. 5300 system - 48 capillary; Fill rows A-D of buffer plate

   2.3. 5300/5400 system - 96 capillary; Fill all rows of buffer plate

3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

   3.1. 5200 system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B "

   3.2. 5300 system - 48 capillary; Fill rows A-D of a sample plate with 100 µL/well, place in drawer '3'

   3.3. 5300/5400 system - 96 capillary; Fill all rows of a sample plate with 100 µL/well, place in drawer '3'

      3.3.1. 5400 system; place in drawer "S"

4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

   4.1. 5200 system; Fill row A of sample plate

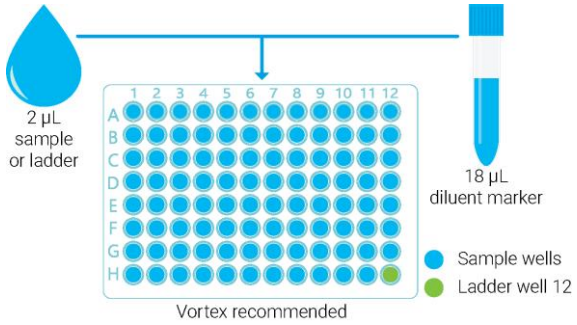
   4.2. 5300 system - 48 capillary; Fill rows A-D of sample plate

   4.3. 5300/5400 system - 96 capillary; Fill all rows of sample plate

5. Dilute the received RNA Ladder aliquot to a working solution of 2 ng/µL.*
   *Initial step done when using as received HS RNA Ladder aliquot for the first time only. Proceed to Step 6 if already completed.

6. Heat denature samples and HS RNA Ladder at 70C for 2 minutes, immediately cool to 4C and keep on ice before use.

7. Mix samples or Ladder with Diluent Marker in sample plate, add 20 µL of BF-25 Blank Solution to unused wells. Place ladder in corresponding well dependent on the capillary size.



5200 system; Ladder – well 12, depending on which row is chosen

5300 system - 48 capillary; Ladder – well D12 or H12, depending on which group is chosen

5300/5400 system - 96 capillary; Ladder – well H12

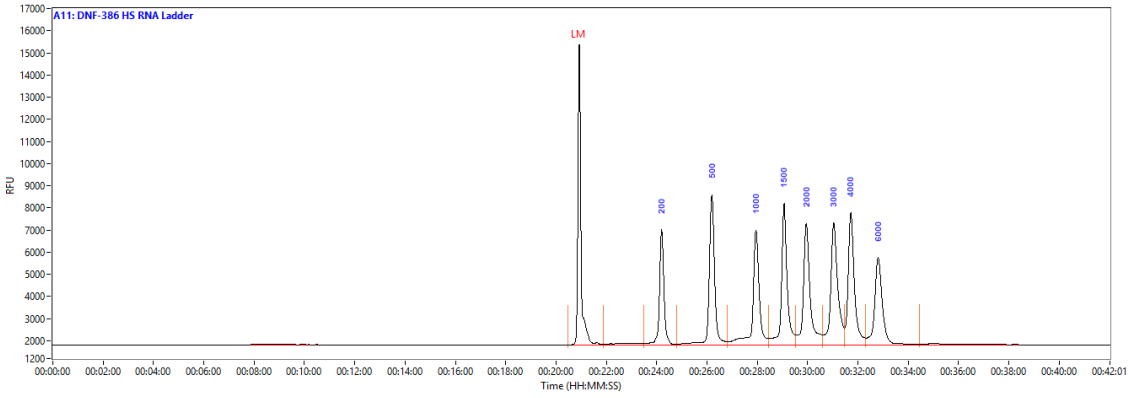
WARNING Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- Refer to product material safety datasheets for further chemical and biological safety information.
- Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Agilent Fragment Analyzer software operating procedure

1. Select Row, Group or Tray to run.
2. Enter **sample ID** and **Tray ID** (optional).
3. Select **Add to Queue**, from the dropdown menus select the corresponding method based on your capillary length;
   - 3.1 DNF-472M22 – HS mRNA 15nt
   - 3.2 DNF-472T22 – HS Total RNA 15nt
   - 3.3 DNF-472M33 – HS mRNA 15nt
   - 3.4 DNF-472T33 – HS Total RNA 15nt
   - 3.5 DNF-472M55 – HS mRNA 15nt
   - 3.6 DNF-472T55 – HS Total RNA 15nt
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

RNA Ladder



Representative HS RNA Ladder result using Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt). Method: **DNF-472T33**. Peaks annotated by size (nt).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-472 HS RNA kit (15 nt) and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
Sample and/or ladder signal too weak or degraded.	1 Sample and/or ladder degraded. 2 Diluent marker degraded. 3 Sample, ladder and/or diluent marker are contaminated. 4 Sample concentration is too low and out of range. 5 Sample not added to Diluent Marker solution or not mixed well. 6 Rinse buffer is not fresh or a wrong rinse buffer is used. 7 Array was contaminated.	1 Use fresh sample and/or ladder. 2 Make sure the diluent marker is stored at -20ºC and keep on ice before use. Use a new vial of diluent marker. 3 Clean working area and equipment with RNaseZap. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot, and diluent marker. 4 Verify sample was within concentration range specified for the HS RNA kid (15 nt). Prepare sample at higher concentration; OR Repeat experiment using increased injection time and/or injection voltage. 5 Verify sample was correctly added and mixed to sample well. 6 Prepare a new rinse buffer plate with 240 µL/well 0.25x TE buffer. 7 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for details).
Sample signal drops abruptly at the end of separation.	1 Separation concentration too high and out of range.	1 Verify sample was within concentration range specified for the HS RNA kit (15 nt).
Missing 25S or 28S ribosomal peak; missing 6000 nt fragment in ladder.	1 No rinse buffer in Marker plate row A; wrong rinse buffer. 2 Dirty array inlet. 3 Aging array.	1 Use a fresh rinse buffer plate with 240 µL/well 0.25x TE buffer. 2 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for details). 3 Replace the array with a new array, if issue persists, contact Agilent Technical Support.
Split RNA peak.	1 Sample's salt concentration was too high.	1 Take steps to lower the salt content in the sample and repeat experiment.

Peak too broad, signal too low and/or migration time too long.	1 Capillary array needs to be reconditioned. 2 Capillary array vent valve is clogged.	1 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for details). 2 Clean vent valve with deionized water (See Fragment Analyzer User Manual for details).
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of the sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20µL is required. 3 Capillary is plugged.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 2 Verify proper volume of solution was added to sample well 3 Check waste plate for liquid in the capillary well. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array.

For Research Use Only

Not for use in Diagnostic Procedures.

Technical Support and Further Information

For technical support, please visit www.agilent.com. It offers useful information and support about the products and technology.

www.agilent.com

Edition 02/22

SD-AT000132