

Agilent DNF-470 Small RNA Kit

Quick Guide

For Research Use Only. Not for use in diagnostic procedures

This Quick Guide is intended for use with the Agilent 5200, 5300, and 5400 Fragment Analyzer systems only. The DNF-470 Small RNA kit is designed for the quantification of small RNA samples, and determination of microRNA region content. Synthetic RNA can be analyzed within the defined sizing region with this kit.

Specifications

Analytical Specifications ^{1,2}	Small RNA Kit
Sizing Range	15 nt – 200 nt
Qualitative Range ¹	25 pg/μL – 2,500 pg/μL
Quantitative Range ¹	50 pg/μL – 2000 pg/μL
Quantification Precision	25% CV (Small RNA Ladder)

Physical Specifications

Total Electrophoresis Run Time	22cm ² : 18 minutes, 33cm: 24 minutes, 55cm: 45 minutes
Samples Per Run	12, 48 or 96; depending on the instrument type
Sample Volume Required	2 μL
Guaranteed Shelf Life	4 months

Recommended Sample Concentrations

MicroRNA	50 pg/μL – 2000 pg/μL
Small RNA	1 ng/μL – 20 ng/μL
Total RNA	5 ng/μL – 100 ng/μL

¹ Results using total RNA, small RNA and microRNA samples and fragments diluted in nuclease-free water.

² The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

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Kit Components – 275 Sample Kit – Refer to product label for proper storage conditions

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6571*		Small RNA, 275, 4 °C	
	DNF-262-0250	Small RNA Separation Gel, 250 mL	1
	DNF-300-0008	BF-25 Blank Solution, 8 mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-470-FR*		Small RNA, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-368-0004	Small RNA Diluent Marker, 4 mL	2
	DNF-361-U060	Small RNA Ladder, 60 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 Solution, 50 mL	1

*Not orderable.

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications. Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

Additional Material Required for Analysis with Fragment Analyzer Systems (not supplied)

Instrument	Compatible Arrays	Part Number
5200 Fragment Analyzer	FA 12 Capillary Array Ultrashort	A2300-1250-2247
	FA 12 Capillary Array Short	A2300-1250-3355
	FA 12 Capillary Array Long	A2300-1250-5580
5300 Fragment Analyzer.	FA 48 Capillary Array Short	A2300-4850-3355
	FA/ZAG 96 Capillary Array Short	A2300-9650-3355
	FA/ZAG 96 Capillary Array Long	A2300-9650-5580
5400 Fragment Analyzer	FA/ZAG 96 Capillary Array Short	A2300-9650-3355
	FA/ZAG 96 Capillary Array Long	A2300-9650-5580

Software

- Fragment Analyzer controller software
- ProSize data analysis software

Reagents

- Capillary Storage Solution (GP-440-0100)

Additional equipment required (not supplied)

- 96-well PCR sample plates (*Refer to Appendix in Fragment Analyzer User Manual*)
- Multichannel pipettor and/or liquid handling device capable of dispensing 1-100 µL (sample plates) and 1,000 µL (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge
- Sub-micron filtered DI water system: for dilutions
- 96-deepwell 1 mL plate: inlet buffer and/or waste plate (Agilent #P60-20 or Fisher Scientific #12-566-120)
- Reagent reservoir 50 mL: for use in pipetting inlet buffer plates (VWR #89094-680, or similar)
- Conical centrifuge tubes for prepared separation gel+dye mixture and/or 1x Capillary Conditioning Solution
 - 50 mL for 5200 Fragment Analyzer system (BD Falcon #352070, Fisher Scientific #14-432-22 or VWR #21008-940)
 - 250 mL for 5300 and 5400 Fragment Analyzer systems (Corning #430776, Fisher Scientific #05-538-53 or VWR #21008-771)
- Vortexer
- RNase-free/nuclease free water
- RNaseZap (Ambion #AM9782 or equivalent product)
- Thermal cycler

WARNING**Working with Chemicals**

- 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 personal protective equipment (PPE).

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Essential Measurement Practices

Environmental conditions	- Ambient operating temperature: 19 – 25 °C (66 – 77 °F) - Keep instrument reagents at room temperature during sample preparation
Sample Input Concentration	- Ensure sample input concentrations lie within kit specifications. - Sample signal should not exceed 60,000 RFU.
Working with RNA	- Ensure all working areas, reagents and plastic ware are RNase free. - Handle RNA samples with care. - Wear gloves at all times. - Thaw RNA samples on ice. - Vortex and centrifuge all samples before use. - Store RNA samples on ice throughout the preparation and analysis procedure.
Steps before sample preparation	- Allow instrument reagents to equilibrate at room temperature for 30 min prior to use - Thaw Small RNA ladder on ice prior to use. - Keep Small RNA Diluent Marker on ice before use. Vortex the tube briefly to mix the contents.
Pipetting practice	- Pipette reagents against the side of the 96-well sample plate or sample tube - Ensure no sample or Diluent Marker remains within or on the outside of the tip
Small RNA Ladder Preparation	- Prior to first use, the Small RNA Ladder should be aliquoted to minimize the number of freeze/thaw cycles. Using the provided Eppendorf LoBind 0.5 mL tube, aliquot 12 µL of Small RNA Ladder per tube into 5 tubes and store the aliquots at less than -70°C. Each aliquot is good for 5 freeze/thaw cycles. - Thaw a Small RNA Ladder aliquot on ice. - Transfer a volume of the Small RNA Ladder for one day of use to an RNase-free PCR tube. Heat-denature the Small RNA Ladder at 70°C for 10 min using a thermal cycler, immediately cool to 4°C and keep on ice before use. - The Small RNA Ladder must be run in parallel with the samples for each experiment to ensure accurate quantification.
RNA Sample Preparation	If desired, heat-denature RNA samples at 70°C for 10 minutes if less than 200 nt or for 70°C for 2 minutes if its total RNA and immediately cool to 4°C. Keep on ice before use.
Sample Plate Preparation	- Using a fresh Rnase-free 96-well sample plate, pipette 18 µL of the Diluent Marker (DM) solution to each well in a row that is to contain sample or Small RNA Ladder. - Fill any unused wells within the row of the sample plate with 20 µL blank solution. - Pipette 2 µL of each RNA sample into the respective wells of the sample plate; mix contents of the well using the pipette by aspiration/expulsion in the pipette tip. - Pipette 2 µL of denatured Small RNA Ladder into the 18 µL of DM solution in the designated ladder well.

Mixing and centrifugation recommendations

- When mixing sample with DM, mix the contents of the well thoroughly. It is suggested to perform **one** of the following methods to ensure complete mixing:
 - After adding 2 μ L of sample or ladder to the 18 μ L of DM, place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm for 2 min. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells.
 - After adding 2 μ L of sample or ladder to the 18 μ L of DM, use a separate pipette tip set to a larger 18 μ L volume, and pipette each well up/down to further mix.
 - Use an electronic pipettor capable of mixing a 10 μ L volume in the tip after dispensing the 2 μ L sample or ladder volume.
- Fill any unused wells within the row of the sample plate with 20 μ L Blank Solution.
- After mixing, centrifuge the plate to remove any air bubbles.
- Run samples immediately after preparation, or within a day with oil overlay. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate.

Gel Preparation

Centrifuge dye prior to opening the vial to reduce risk of leaking. Ensure the gel + dye is mixed without generating bubbles, gently invert tube 5-10 times.

Number of Samples	Intercalating Dye Volume (μ L)	Separation Gel Volume (mL)
12	1	10
24	1.5	15
48	2.5	25
96	4.5	45
192	8	80
384	16	160

Conditioning Solution

The provided 5X Conditioning Solution must be diluted to 1X using submicron DI water prior to use. Invert to mix.

Number of Samples	Volume of 1X Conditioning Solution (mL)
12	10
24	15
48	25
96	45
192	80
384	160

Agilent Small RNA DNF-470 Kit Operating Procedure

1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1X Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer 'W'.
4. Place a fresh 1X Inlet Buffer, 1 mL/well, in drawer "B". Replace daily.
 - 5200 – row A
 - 5300 – 48 capillary, rows A-D
 - 5300/5400 – 96 capillary, all rows

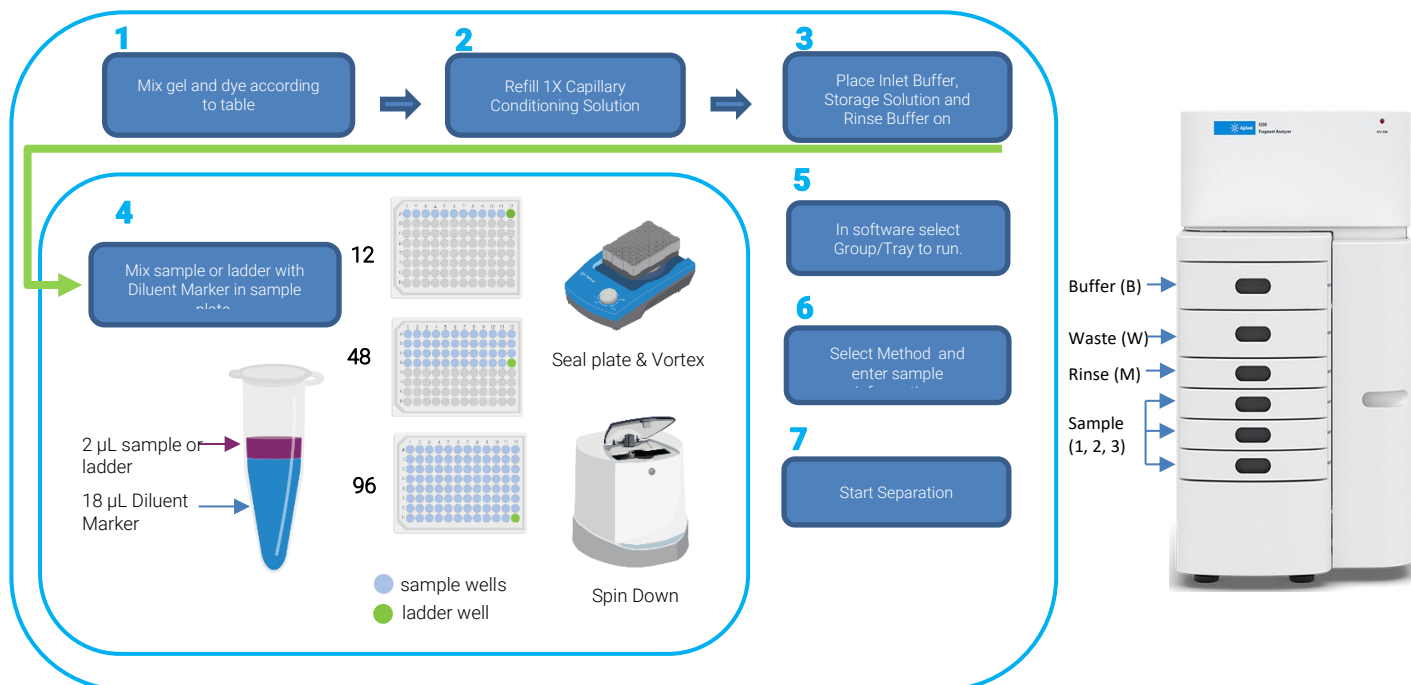
Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.

 - 5200 – row H, 1 mL/well, drawer B
 - 5300 – 48 capillary, rows A-D, 100 µL/well, drawer 3
 - 5300/5400 – 96 capillary, all rows, 100 µL/well, drawer 3

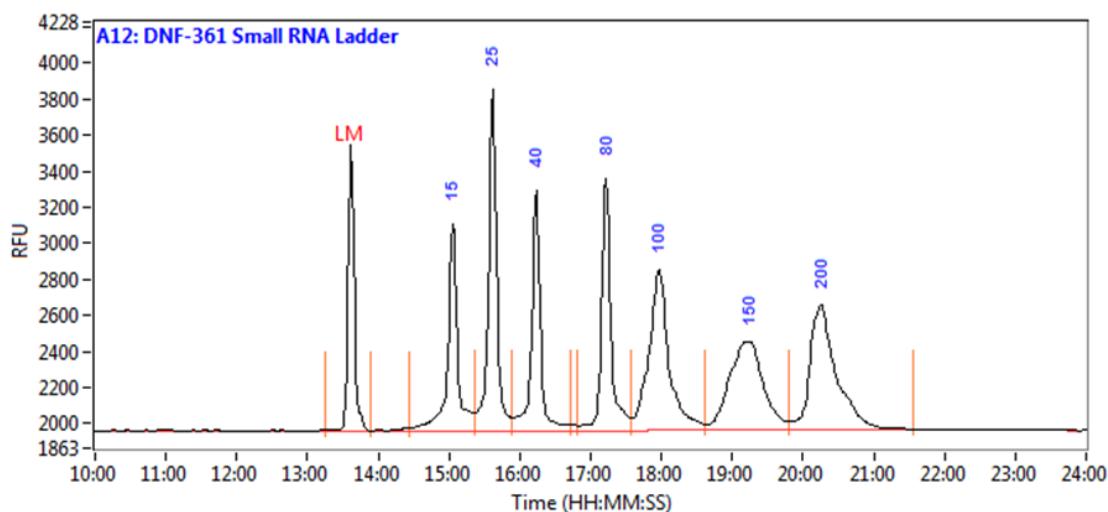
Place 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M". Replace daily.

 - 5200 – Row A
 - 5300 – 48 capillary, rows A-D
 - 5300/5400 – 96 capillary, all rows
5. Mix samples or ladder with diluent marker in sample plate, add 20 µL of Blank Solution to unused wells. Place ladder in corresponding well (see sample plate image below), depending on capillary array used.
6. Select Row/Group/Tray to run. Enter sample ID and Tray ID, if desired.
7. Add to queue, from the dropdown select the corresponding method based on your capillary length;
 - DNF-470-22
 - DNF-470-33
 - DNF-470-55

Enter Tray Name, Folder Prefix and Notes, if desired.
8. Add method to the queue by selecting "OK", press play  to start the separation.



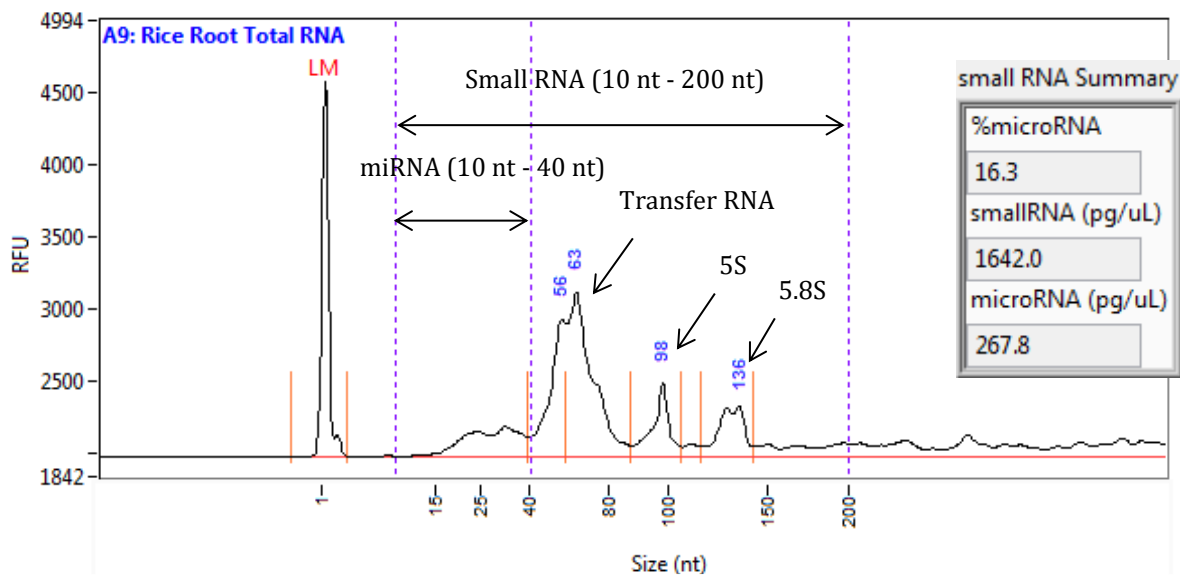
Small RNA Ladder result



Representative Small RNA Ladder result using the Fragment Analyzer system with the DNF-470 Small RNA kit. Peaks annotated by size (nt). Method: DNF-470-33. RFU values may differ between instruments.

Small RNA Sample

Figure shows the typical results for a RNA sample with peaks annotated by size in nt. The data was normalized to the lower marker and the size was calibrated to the Small RNA Ladder run in parallel to the sample. The micro RNA (miRNA) size region and the small RNA size region are set to default values of 10 nt – 40 nt and 10 nt – 200 nt, respectively, and marked with vertical dashed cursors. These regions can be adjusted in the ProSize data analysis software by the user if desired. The **smallRNA (pg/μL)** concentration, **microRNA (pg/μL)** concentration, and **%microRNA** values (percentage miRNA in the small RNA region) are reported for each sample.



Rice root RNA sample result using the Fragment Analyzer system with the DNF-470 Small RNA Analysis Kit. The miRNA and small RNA sizing ranges are adjustable by user in the ProSize data analysis software. Method: DNF-470-33. Peaks annotated by size (nt).

Troubleshooting

The following table lists several potential kit specific issues which may be encountered when using the DNF-470 Small RNA kit 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 Sample, ladder and/or diluent marker are contaminated. 3 Sample concentration is too low and out of range. 4 Sample not added to Diluent Marker solution or not mixed well. 5 Rinse buffer is not fresh or a wrong rinse buffer is used. 6 Array was contaminated.	1 Use fresh sample and/or ladder. 2 Clean working area and equipment with RNaseZap. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot, and diluent marker. 3 Verify sample was within concentration range specified for the Small RNA Kit and prepare sample at higher concentration. 4 Verify sample was correctly added and mixed to sample well. 5 Prepare a new rinse buffer plate with 200 µL/well 0.25x TE buffer. 6 Follow Method C outlined in the Fragment Analyzer System User Manual to decontaminate and clean the capillary array.
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 Small RNA kit and dilute with RNase-free water as needed.
Missing LM signal or noisy baseline	1 Expired DM solution 2 Dirty array inlet. 3 Aging array.	1 Use a fresh DM solution. 2 Follow Method C outlined in the Fragment Analyzer System User Manual to clean the array. 3 Replace the array with a new array, if issue persists, contact Agilent Technical Support.
Split RNA peak	1 Samples salt concentration was too high 2 Sample was not heat denatured.	1 Take steps to lower the salt content in the sample and repeat experiment. 2 Heat denature sample/ladder plate at 70°C for 2 minutes.
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 Follow Method C outlined in the Fragment Analyzer System User Manual to clean the array. 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 20 µL 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 using a 96-deepwell plate. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array.

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Technical Support and Further Information

For technical support please visit www.agilent.com which offers useful information and support regarding the products and technology.

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