Agilent DNF-470 Small RNA Kit Quick Guide for 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. The Small RNA Kit (275 samples) (Part # DNF-470-0275) is designed for the sizing and quantification of Small RNA samples, and determination of microRNA region content. Synthetic RNA can also be analyzed within the defined sizing region with this kit.

Specifications

Analytical specifications[1,2]	Small RNA assay
Sizing Range	15 nt – 200 nt
Qualitative Range	25 pg/µL – 2500 pg/µL (microRNA region)
Quantitative Range	50 pg/µL – 2000 pg/µL
Quantification Precision	25% CV (Small RNA Ladder)
Physical Specifications[3]	
Total electrophoresis run time	22cm[2]: 18 minutes, 33cm: 24 minutes
Samples per run	12, 48 or 96; depending on the instrument type[1]
Sample volume required	2 µL
Kit stability	4 months
Recommended Sample Concentrations	
MicroRNA	50 pg/µL – 2000 pg/µL 10-40nt
Small RNA	1 ng/µL – 20 ng/µL 10-200n
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 22 cm effective, 47 cm total length capillary array is only available for 12-capillary Fragment Analyzer instruments.

Kit Components – 275 Sample Kit

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, 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-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, 600 µL	1
5191-6612*		Quantitative DNA, RT	
	C275-130	Eppendorf LoBind 0.5mL tubes (bag of 50)	1
	DNF-475-0050	5x Capillary Conditioning Soln, 50mL • 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.

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

Small RNA Diluent Marker Preparation

- Remove the Small RNA Diluent Marker from -20°C and keep it on ice before use. Vortex the tube briefly to mix the content. Spin the tube after mixing to ensure liquid is at the bottom of the tube.

Small RNA Ladder Preparation

- Prior to first use, the Small RNA Ladder solution should be aliquoted to minimize the number of freeze/thaw cycles. Using the provided Eppendorf LoBind 0.5 mL tubes, 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.

RNA Sample Preparation

- It is recommended to heat-denature all RNA samples at 70°C for 10 min and immediately cool to 4°C and keep on ice before use.
- If the concentration of the input sample is above the recommended range, dilute the sample with RNase-free water.

Sample Plate Preparation

- Using a fresh RNase-free 96-well sample plate, pipette 18 µL of the Small RNA 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/well of BF-25 Blank Solution.
- Pipette 2 µL of each denatured RNA sample into the respective wells of the sample plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
- Small RNA Ladder: The Small RNA Ladder must be run in parallel with the samples for each experiment to ensure for accurate quantification. Pipette 2 µL of denatured Small RNA Ladder into the 18 µL of Diluent Marker (DM) Solution in Well 12 of each row to be analyzed. Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
- After mixing sample or Small RNA Ladder with Small RNA Diluent Marker 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.

- 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 2-8°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.
- To run the samples, place the plate in one of the three sample plate trays (Drawers 4-6 from the top) of the Fragment Analyzer instrument. Load or create the experimental method.

  Important Sample Mixing Information:

  When mixing sample with diluent marker, 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 18 µL of diluent marker, swirl the pipette tip while pipetting up/down to further mix. OR
  - After adding 2 µL of sample or ladder to 18 µ µL of diluent marker, place a plate seal on the sample plate and vortex the sample plate at 3,000 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. OR
  - When adding 2 µL of sample or ladder to 18 µL of diluent marker, use a separate pipette tip set to a larger 18 µL volume, and pipette each well up/down to further mix. OR
  - 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.

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.

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.~~

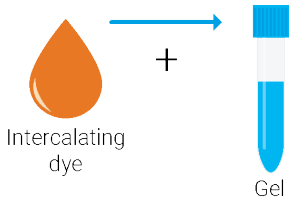
~~**5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays**~~

~~# 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 Small RNA DNF-470 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"~~

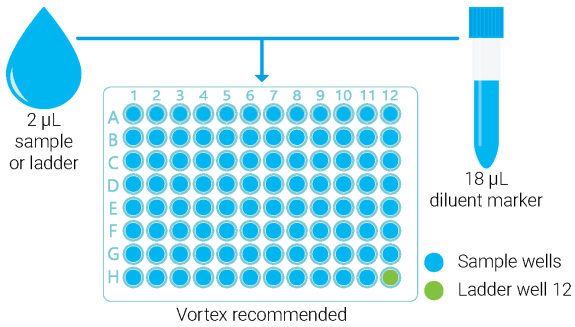
4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

   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. 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

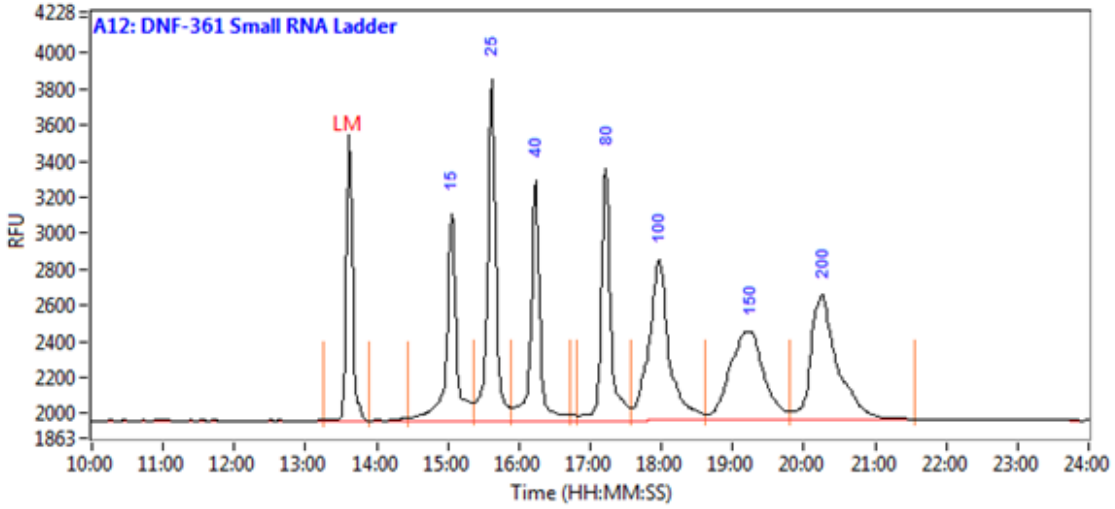
The handling of reagents and chemicals might hold health risks.

- 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-470-22 – Small RNA
   - 3.2 DNF-470-33 – Small RNA
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

Small RNA Ladder result



Representative Small RNA Ladder result using Fragment Analyzer system with the DNF-470 Small RNA Analysis kit. Method: DNF-470-33. Peaks annotated by size (nt).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered on rare occasion when using the Small RNA Analysis 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.	1 Use fresh sample and/or ladder.
	2 Sample, ladder and/or diluent marker are contaminated.	2 Clean working area and equipment with RNaseZap. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot and diluent marker.
	3 Sample concentration is too low and out of range.	3 Verify sample was within concentration range specified for the Small RNA Analysis kit and prepare sample at high concentration; OR Repeat experiments using increased injection time and/or injection voltage.
	4 Sample not added to Diluent Marker solution or not mixed well	4 Verify sample was correctly added and mixed to sample well.
	5 Rinse buffer is not fresh or a wrong rinse buffer used.	5 Prepare a new rinse buffer plate with 200 µL/well 0.25x TE Rinse Buffer.
	6 Array was contaminated.	6 Follow the Method C – outlined in Capillary Array Cleaning of the Fragment Analyzer User Manual to decontaminate and clean the capillary array.
Sample signal drops abruptly at the end of separation.	1 Sample concentration too high and out of range.	1 Verify sample was within concentration range specified for the Small RNA Analysis kit.
Missing LM signal or noisy baselilne.	1 Expired Diluent Marker solution.	1 Use a fresh Diluent marker solution.
	2 Dirty array inlet.	2 Follow Method C – outlined in Capillary Array Cleaning of the Fragment Analyzer User Manual to clean the array.
	3 Aging array.	3 Replace the array with a new array. If issue persists, contact Agilent Technical Support.

Technical Support and Further Information

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

www.agilent.com

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