Agilent DNF-474 HS NGS Fragment 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. The DNF-474 HS NGS Fragment assay is designed for the quantitative and qualitative analysis of NGS libraries and their intermediates from 100 to 6,000 bp.

Specifications

Analytical specifications[1,2]	HS NGS Fragment assay
Sizing Range	100 bp – 6,000 bp
Sizing Accuracy[2]	± 5% or better
Sizing Precision[2]	2% CV
Separation Resolution	100 bp – 1,000 bp ≤ 5% 2,000 bp – 6,000 bp ≤ 10%
Fragment Concentration Range[2]	5 pg/µL – 500 pg/µL input DNA
Smear Concentration Range	50 pg/µL – 5,000 pg/µL input DNA
Quantification Accuracy[2,3]	± 25%
Quantification Precision[2,3]	15% CV
Quantitative Range	Fragment[3]:5 - 500 pg/µL; Smear[3]:50 – 5,000 pg/µL
Physical Specifications	
Total electrophoresis run time	22cm[1]: 25 minutes, 33cm: 50 minutes, 55cm: 80 minutes
Samples per run	12, 48 or 96; depending on the instrument type
Sample volume required	2 µL
Kit stability	4 months

1 The FA 12-Capillary Array Ultrashort, 22 cm is only available for 5200 Fragment Analyzer system.
2 Results using DNA ladder in 1X TE buffer.
3 Results using DNA samples in 1X TE buffer.

Kit Components – 500 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6578*		HS NGS Fragment (1-6000bp), 500, 4°C	
	DNF-240-0240	NGS Separation Gel, 240 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-474-FR*		HS NGS Frag (1-6000bp), -20°C	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-373-0003	HS NGS Diluent Marker (1-6000bp), 2.4 mL	5
	DNF-396-U100	HS NGS DNA Ladder, 100 µL	1
DNF-475-0050	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
5191-5679*		HS NGS Fragment (1-6000bp), 1000, 4°C	
	DNF-240-0500	NGS Separation Gel, 500 mL	1
	DNF-300-0008	BF-25 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-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-476-FR*		HS NGS Frag (1-6000bp), -20°C	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	DNF-373-0003	HS NGS Diluent Marker (1-6000bp), 2.4 mL • Lower marker (set to 1 bp) and 6,000 bp upper marker	10
	DNF-396-U100	HS NGS DNA Ladder, 100 µL • Fragments from 100 bp – 3,000 bp; 1 ng/µL total DNA concentration	2
DNF-475-0100	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.

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

Mixing and centrifugation recommendations	• Apply a new seal to 96-well sample plate prior to mixing and centrifugation
	• When mixing sample with Diluent Marker (DM), 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. After mixing, briefly centrifuge and visually confirm that all liquid is collected at the bottom of the 96-well sample plate or tube strips and any air bubble is removed
	  ◦ After adding 2 µL of sample or ladder to the 22 µL of DM, 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.
	  ◦ After adding 2 µL of sample or ladder to the 22 µL of DM, 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 or ladder volume. Some models enable using the pipette tip for both adding and mixing.
	• 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

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.

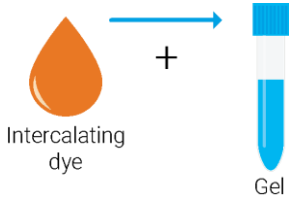
5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays

# 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 NGS Fragment DNF-474 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"

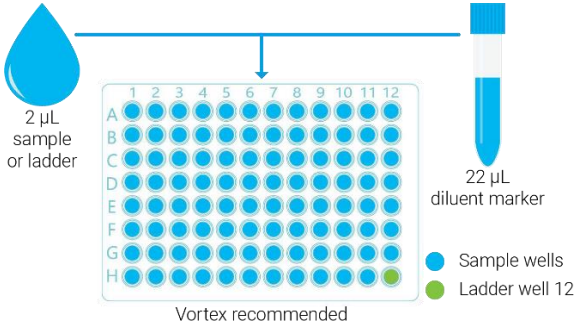
4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

   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 24 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

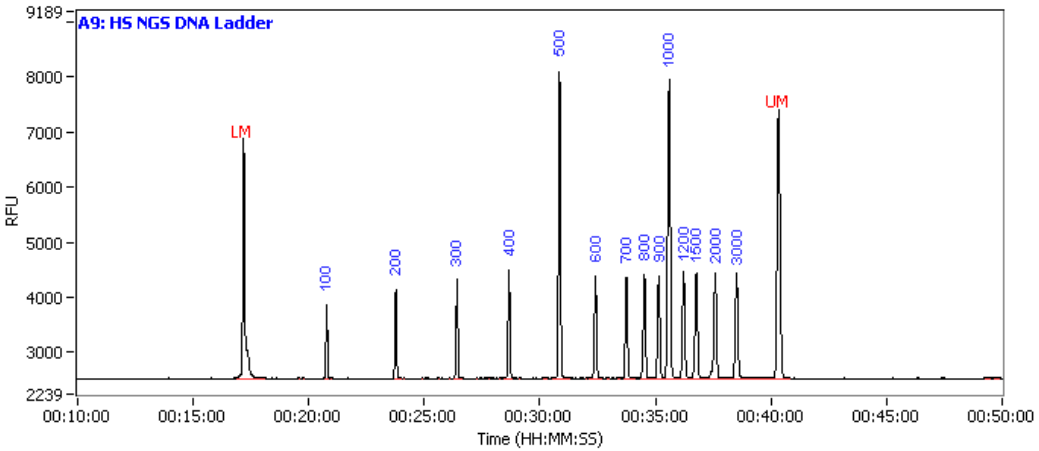
The handling of reagents and chemicals might hold health risks.

- 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-474-22 – HS NGS Fragment 1-6000bp
   - 3.2 DNF-474-33 – HS NGS Fragment 1-6000bp
   - 3.3 DNF-474-55 – HS NGS Fragment 1-6000bp
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

DNA Ladder result



Representative NGS DNA Ladder result using the Fragment Analyzer system with the DNF-474 HS NGS Fragment kit (1bp – 6,000bp). Method: DNF-474-33 (short array). Peaks annotated by size (bp).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the HS NGS Fragment kit (1-6000 bp) (Part #DNF-474) and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1 Input DNA sample concentration is too high. Ensure total signal height does not exceed 20,000 RFU, or total input DNA concentration does not far exceed 5,000 pg/µL.	1 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment; OR Repeat experiment using decreased injection time (e.g., 10 sec); OR Prepare fresh sample using NGS Fragment kit (1-6000 bp) (Part # DNF-473), which covers input DNA range from 5 – 100 ng/µL.
DNA Sample smear overlaps with Lower/Upper Marker peak.	1 Input DNA sample size distribution outside of assay range.	1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment; OR Prepare fresh sample using HS Large Fragment Kit (part #DNF-493)
	2 Input DNA sample concentration too high.	2 Verify sample was correctly added and mixed in sample well.
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.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate.
	2 Insufficient sample volume. A minimum of 20µL is required.	2 Verify proper volume of solution was added to sample well
	3 Capillary is plugged.	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

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