



Agilent DNF-493 HS Large Fragment 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 Large Fragment kit (p/n DNF-493-0500 and DNF-493-1000) is designed for the sizing and quantification of small to mid-range molecular weight dsDNA smears/fragments. Example applications include quality control of Next Generation Sequencing (NGS) libraries.

Specifications

Analytical specifications	HS Large Fragment assay
DNA Sizing Range	75 bp – 20,000 bp
DNA Sizing Accuracy ^{1,2}	± 15% or better
DNA Fragment Concentration Range ¹	0.005 ng/μL – 0.5 ng/μL input DNA
DNA Smear Concentration Range ¹	0.05 ng/μL – 5 ng/μL input DNA
DNA Quantification Accuracy ¹	± 25%
DNA Quantification Precision ¹	20% CV
Maximum DNA Concentration	0.5 ng/μL per fragment; 5 ng/μL per total sample
Physical Specifications ³	
Total electrophoresis run time	22cm ³ : 30 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 Results using DNA Ladder of DNA fragment standards prepared in 1x TE buffer.

² The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments

Kit Components – 500 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6589*		HS Large Fragment, 500, 4°C	
	DNF-220-0240	Large Fragment Separation Gel, 240 mL	1
	DNF-300-0008	BF-25 Blank Solution, 8mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL <ul style="list-style-type: none"> Dilute with sub-micron filtered water prior to use 	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125mL	1
DNF-493-FR*		HS Large Fragment, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-381-0003	HS Large Fragment Diluent Marker, 2.4mL	5
	DNF-379-U100	HS Large Fragment DNA Ladder, 100µL	1
DNF-475-0050		5x Capillary Conditioning Solution, RT <ul style="list-style-type: none"> Dilute with sub-micron filtered water prior to use 	1

Kit Components – 1000 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6590*		HS Large Fragment, 1000, 4°C	
	DNF-220-0500	HS Large Fragment Separation Gel, 500 mL	1
	DNF-300-0008	BF-25 Blank Solution, 8mL	1
	DNF-355-0300	5x 930 dsDNA Inlet Buffer, 300 mL <ul style="list-style-type: none"> Dilute with sub-micron filtered water prior to use 	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125mL	1
DNF-492-FR*		HS Large Fragment, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	DNF-381-0003	HS Large Fragment Diluent Marker, 2.4mL	10
	DNF-379-U100	HS Large Fragment DNA Ladder, 100µL	2
DNF-475-0100		5x Capillary Conditioning Solution, RT <ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> • Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
Steps before sample preparation	<ul style="list-style-type: none"> • Allow reagents to equilibrate at room temperature for 30 min prior to use
Pipetting practice	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 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

¹One sample well per separation is dedicated to the ladder.

²A 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

¹One sample well per separation is dedicated to the ladder.

²A 5 mL minimum volume in the tube is included.

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

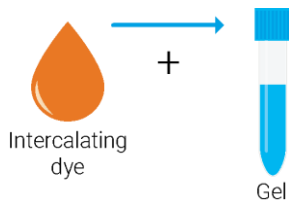
# of Samples to be Analyzed ¹	Volume of Intercalating Dye	Volume of Separation Gel ²	Volume of 1x Conditioning Solution ²
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

¹One sample well per separation is dedicated to the ladder.

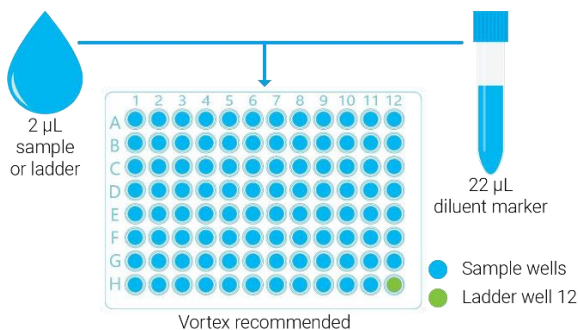
²A 5 mL minimum volume in the tube is included.

Agilent HS Large Fragment DNF-493 assay operating procedure

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



- Place a fresh 1x 930 dsDNA Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.
 - 5200 system; Fill row A of buffer plate
 - 5300 system - 48 capillary; Fill rows A-D of buffer plate
 - 5300/5400 system - 96 capillary; Fill all rows of buffer plate
- Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.
 - 5200 system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B"
 - 5300 system - 48 capillary; Fill rows A-D of a sample plate with 100 μ L/well, place in drawer '3'
 - 5300/5400 system - 96 capillary; Fill all rows of a sample plate with 100 μ L/well, place in drawer '3'
 - 5400 system; place in drawer "S"
- Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 μ L/well. Replace daily.
 - 5200 system; Fill row A of sample plate
 - 5300 system - 48 capillary; Fill rows A-D of sample plate
 - 5300/5400 system - 96 capillary; Fill all rows of sample plate
- Mix samples or Ladder with Diluent Marker in sample plate, add 24 μ L 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

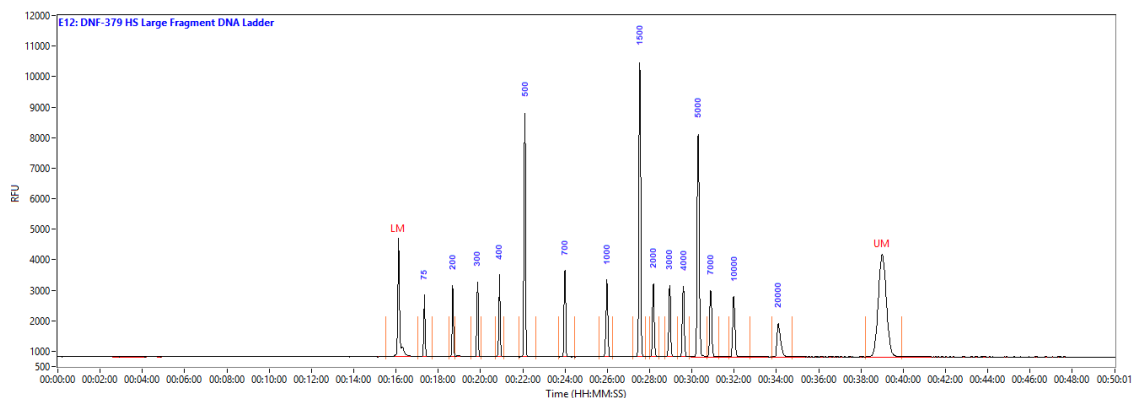
The handling of reagents and chemicals might hold health risks.

- 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-493-22– HS Large Fragment
 - 3.2 DNF-493-33 – HS Large Fragment
 - 3.3 DNF-493-55 – HS Large Fragment
4. Enter **Tray Name**, **Folder Prefix**, and **Notes**(optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

HS Large Fragment Ladder result



Large Fragment DNA Ladder result using the Fragment Analyzer system with the HS Large Fragment Kit. Peaks annotated by size (bp).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-493 HS Large Fragment Kit 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 too high. Ensure peak height does not exceed 2,000 RFU (smear) or 20,000 RFU (fragment), or total input concentration does not exceed recommended limits.	1 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment OR Repeat experiment using decreased injection time or voltage.
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
	2 Input DNA sample concentration too high.	2 Repeat experiment using DNF-488 High Sensitivity gDNA Analysis Kit (uses lower marker only).
No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1 Sample concentration too low and out of range.	1 Prepare more concentrated sample and repeat experiment: OR repeat experiment using increased injection time and/or injection voltage.
	2 Sample not added to Diluent Marker solution or not mixed well.	2 Verify sample was correctly added and mixed to sample well.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of 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 20 µL 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 Appendix – Capillary Array Cleaning of the Fragment Analyzer User 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, support and current developments about the products and technology.

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