Agilent DNF-464 HS Large 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 HS Large Fragment 50 kb kit (500 Samples) (Part # DNF-464-0500) is designed for the sizing and quantification of medium to high molecular weight dsDNA smears/fragments. Example applications include quality control of long read Next Generation Sequencing (NGS) libraries (e.g., PacBio).

Specifications

Analytical specifications[1,2]	HS Large Fragment assay
DNA Sizing Range	75 bp – 48,500 bp
DNA Sizing Accuracy[1]	± 15% or better
DNA Fragment Concentration Range[2]	5 pg/µL – 600 pg/µL input DNA (optimal concentration 500 – 600 pg/µL)
DNA Smear Concentration Range[2]	50 pg/µL -5 ng/µL input DNA (optimal concentration of 1 ng/µL)
DNA Quantification Accuracy[2]	± 25%
DNA Quantification Precision[2]	20% CV
Maximum DNA Concentration	600 pg/µL per fragment; 5 ng/µL per total sample
Physical Specifications[3]	
Total electrophoresis run time	22cm[3]: 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 Results using DNA Fragment standards at 600 pg/µL and DNA smears at 1 ng/µL prepared from 1x TE buffer.
2 Results using DNA Fragment standards and DNA smears prepared from 1x TE buffer.
3 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
5191-6568*		HS Large Fragment 50kb, 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 • Dilute with sub-micron filtered water prior to use	1
	DNF-365-U125	HS Extended Large Frag DNA Ladder, 125mL	1
	DNF-495-0060	Dilution Buffer 1X TE, 60mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-464-FR*		HS LRG Fragment 50kb, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-381-0003	HS Large Fragment Diluent Marker, 2.4 mL	5
5191-6612*		Qualitative DNA, RT	
	C275-130	Eppendorf LoBind 0.5 mL tubes (bag of 50)	1
	DNF-475-0050	5x Capillary Conditioning Soln, 50 mL	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: 1. The Lambda DNA fragment (48,500 bp) in the HS Extended Large Frag DNA Ladder is sensitive to degradation. The Ladder should be kept at 2-8°C. Do not freeze or subject to repetitive freeze/thaw cycles. Do not pipette the ladder up and down; vortex with care.

2. The Large Fragment Diluent Marker (DM) solution is provided in aliquots of 2.4 mL vials. To minimize the number of freeze/thaw cycles, it is highly recommended to work with only one aliquot of DM solution at a time. The DM solution is light and temperature sensitive. For maximum performance, the DM solution should be kept frozen at –20°C and protected from light when not in use. The DM solution should NOT be left at room temperature longer than 1 h at a time for sample preparation.

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.

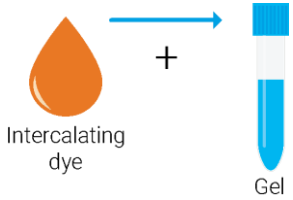
5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays

# 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 Large Fragment DNF-464 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"

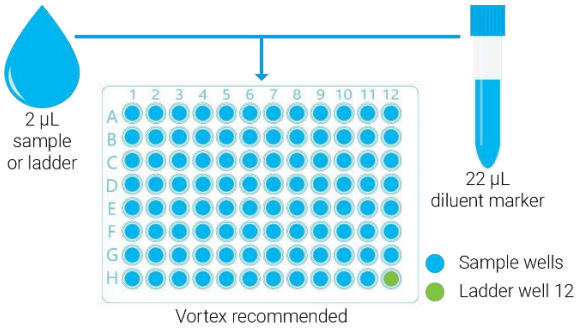
4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

   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 – 12 capillary: 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

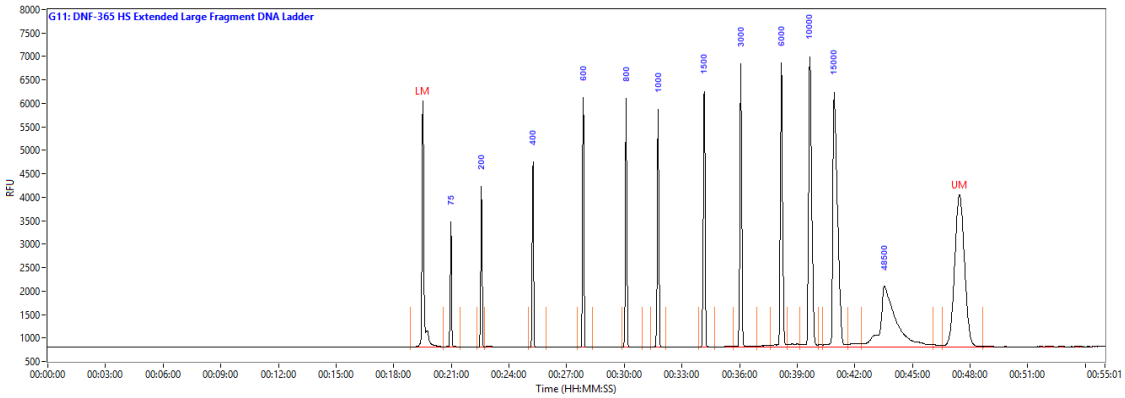
The handling of reagents and chemicals might hold health risks.

- 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-464-22 – HS Large Fragment 50kb
   - 3.2 DNF-464-33 – HS Large Fragment 50kb
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



High Sensitivity Extended Large Fragment DNA Ladder result, using the Fragment Analyzer system with the DNF-464 High Sensitivity Large Fragment 50 kb kit. Peaks are annotated by size (bp). Method: DNF-464-33 (33cm "short" array).

Appendix A: Maximum Sizing Accuracy Alternative Workflow

The sizing of dsDNA fragments during electrophoresis can be sensitive to sample concentration, with higher concentration samples generally running faster than lower concentration samples. This phenomenon is more pronounced for high molecular weight dsDNA fragments or smears, such as genomic DNA and large fragment NGS libraries used for long read sequencing applications.

To maximize the sizing accuracy and reproducibility of large molecular weight dsDNA samples, and better enable sample to sample sizing comparisons, it is highly recommended to first normalize the sample concentration prior to performing the analysis.

The HS Extended Large Frag DNA Ladder concentration and the method employed in the DNF-464 HS Large Fragment 50 kb kit has been optimized to provide high sizing accuracy for dsDNA smears when the total sample concentration is normalized to a target concentration of 1 ng/ µL prior to analysis.

For dsDNA fragments, a target concentration of 500 – 600 pg/µL is recommended to provide maximum sizing accuracy.

Essential Steps for Maximum Sizing Accuracy: Sample Preparation

1. Measure the total concentration of the DNA sample using a fluorometric, dye-based detection method.
2. Dilute the DNA sample to a target concentration of 1 ng/ µL, using the supplied Dilution Buffer 1x TE (p/n DNF-495-060). DO NOT pre-dilute samples with DI water.
3. Dilute the DNA fragment sample to a target concentration of 500 - 600 pg/ µL, using the supplied Dilution Buffer 1x TE (p/n DNF-495-060). DO NOT pre-dilute samples with DI water.
4. Following normalization of the DNA sample concentration, follow the standard sample plate preparation procedures outlined in the **Error! Reference source not found.** section of this User Manual (2 µL sample added to 22 µL of HS Large Fragment Diluent Marker).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-464 HS Large Fragment 50 kb kit. and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
8,500 bp Lambda DNA fragment in the Ladder is degraded or missing.	1 The Ladder was pipetted up and down excessively.	1 Use a new Ladder aliquot and avoid pipetting the Ladder up and down excessively.
	2 The Ladder was stored inappropriately. The Ladder should be stored at 2-8°C and freeze-thaw cycles avoided.	2 Store and handle the Ladder as directed in this User Manual.
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 supplied Dilution Buffer 1x TE (DNF-495) 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 repeat experiment using DNF-488 HS gDNA 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.
Marker and/or Ladder peaks are	1 Capillary array needs to be	1 Flush array with 0.5 N NaOH

broad, signals are lower than expected, and/or migration time longer than expected,	reconditioned. 2 Capillary array vent valve is clogged.	solution and repeat experiment. 2 Clean vent valve with deionized water. (See User Manual for details).
48,500bp Lambda DNA fragment peak is the Ladder is split and/or not assigned properly in the software.	1 Occasional Ladder lot variations may result in secondary peak appearing before main Lambda DNA peak.	1 Manually delete extra peak migrating before main Lambda DNA peak or increase peak height threshold in ladder well to not call extra peak. NOTE: Sample sizing and quantification will not be affected by the presence of the extra peak, if the Lambda DNA peak is correctly assigned in the Ladder.

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.

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