

Agilent DNF-464 HS Large Fragment 50 kb 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-464 HS Large Fragment 50 kb kit is designed for the sizing and quantification of medium to high molecular weight dsDNA smears/fragments at low sample concentrations.

Specifications

Analytical Specifications ^{1,2}	HS Large Fragment 50 kb Kit
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: 55 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 <15kb 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 FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

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Kit Components – 500 Sample Kit – Refer to product label for proper storage conditions

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6568*		HS Large Fragment 50 kb, 500, 4 °C	
	DNF-220-0240	Large Fragment Separation Gel, 240 mL	1
	DNF-300-0008	BF-25 Blank Solution, 8 mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL	1
	DNF-365-U125	HS Extended Large Fragment DNA Ladder, 125 µL	1
	DNF-495-0060	Dilution Buffer 1X TE, 60 mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-464-FR*		HS Large Fragment 50 kb, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-381-0003	HS Large Fragment Diluent Marker, 2.4 mL	5
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

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 μ L (sample plates) and 1,000 μ L (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge
- Adhesive PCR plate seals
- 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

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).

Essential Measurement Practices

IMPORTANT

- The Lambda DNA fragment (48,500 bp) in the High Sensitivity Extended Large Fragment DNA Ladder is sensitive to degradation. The ladder should be kept at 4°C. Do not pipette the ladder up and down; mix by vortexing, with care. 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 hour at a time for sample preparation.

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.

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Steps before sample preparation	<ul style="list-style-type: none"> Allow instrument reagents to equilibrate at room temperature for 30 min prior to use.
Pipetting practice	<ul style="list-style-type: none"> 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
Ladder handling and storage	<ul style="list-style-type: none"> Before using the kit, aliquot the High Sensitivity Extended Large Fragment DNA Ladder into 10 μL aliquots (12 tubes), using the Eppendorf LoBind® 0.5 mL tubes provided in the kit. Label the aliquots and store at 4°C. Do not store the Ladder frozen. Before use, equilibrate the ladder aliquots to room temperature for about 30 min. Do not pipette the ladder aliquots up and down, nor flick the tube to mix, as this may induce degradation of lambda DNA ladder fragment. Gently vortex the aliquot and spin the ladder tube prior to use. Each 10 μL aliquot is good for 4-times use (2 μL per use).
Maximum sizing accuracy alternative workflow	<ul style="list-style-type: none"> 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 phenomena is more pronounced for high molecular weight dsDNA fragments or smears, such as genomic DNA and large fragment NGS libraries used in 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 High Sensitivity Extended Large Fragment DNA Ladder concentration and the method employed in the DNF-464 High Sensitivity 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.
Mixing and centrifugation recommendations	<ul style="list-style-type: none"> When mixing sample or ladder with diluent marker solution, it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. After adding 2 μL of sample or ladder to the 22 μL of diluent marker, place a plate seal on the sample plate and vortex the sample plate at 3000 rpm for 2 min (we recommend two vortexing pulses, 1 min each). 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. 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 HS Large Fragment DNF-464 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

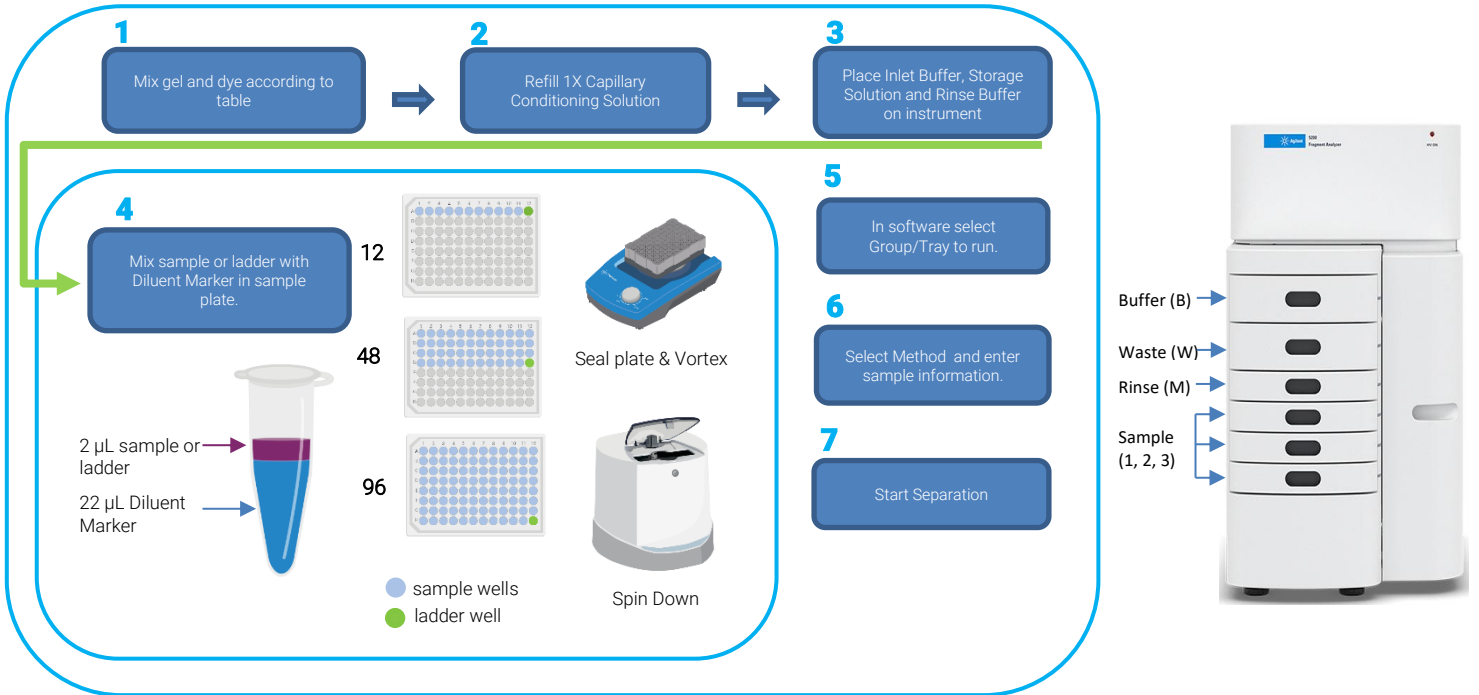
Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.

 - 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

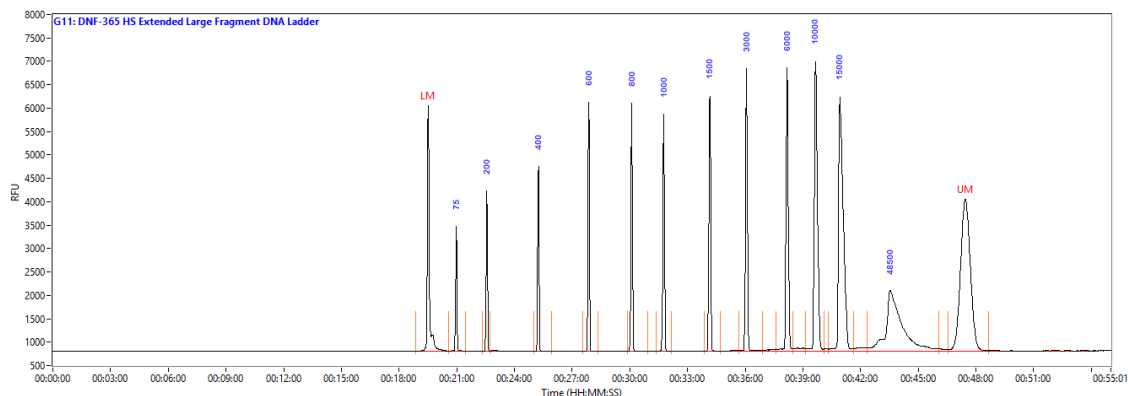
Place 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M". Replace daily.

 - 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 24 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-464-22
 - DNF-464-33
 - DNF-464-55

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



DNA Ladder result



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

Troubleshooting

The following table lists several potential kit 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
48,500 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.
DNA sample smear overlaps with Lower/Upper Marker peak.	1 Input DNA sample size distribution outside of kit range.	1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment; OR repeat experiment using DNF-468 HS Genomic DNA 50 kb 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.
	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.	<ol style="list-style-type: none"> 1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 μL is required. 3 Capillary is plugged. 	<ol style="list-style-type: none"> 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.
Marker and/or Ladder peaks are broad, signals are lower than expected, and/or migration time longer than expected.	<ol style="list-style-type: none"> 1 Capillary array needs to be reconditioned. 2 Capillary array vent valve is clogged. 	<ol style="list-style-type: none"> 1 Flush array with 0.5 N NaOH solution and repeat experiment. 2 Clean vent valve with deionized water. (See Fragment Analyzer User Manual for details).
48,500 bp Lambda DNA fragment peak is the Ladder is split and/or not assigned properly in the software.	<ol style="list-style-type: none"> 1 Occasional Ladder lot variations may result in secondary peak appearing before main Lambda DNA peak. 	<ol style="list-style-type: none"> 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.

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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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