# 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 kit is designed for the sizing and quantification of medium to high molecular weight dsDNA smears/fragments at low sample concentrations.

## Specifications

| Analytical Specifications[1,2,3] | HS Large Fragment Kit |
|---|---|
| DNA Sizing Range | 75 bp – 48,500 bp |
| DNA Sizing Accuracy[1,2] | ± 15% at ≤15 Kb[1]; ± 25% ≥15Kb[2] |
| DNA Sizing Precision | ± 10% |
| DNA Fragment Concentration Range[3] | 5 pg/µL – 600 pg/µL input DNA (optimal concentration 500 – 600 pg/µL) |
| DNA Smear Concentration Range[3] | 50 pg/µL – 5 ng/µL input DNA (optimal concentration of 1 ng/µL) |
| DNA Quantification Accuracy[3] | ± 25% |
| DNA Quantification Precision[3] | 20% CV |
| Maximum DNA Concentration | 600 pg/µL per fragment; 5 ng/µL per total sample |
| **Physical Specifications[4]** | |
| Total Electrophoresis Run Time | 22cm[4]: 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 |
| Guaranteed Shelf Life | 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 at >15kb at 600 pg/µL and DNA smears at 1 ng/µL prepared from 1X TE buffer
3 Results using DNA Fragment standards and DNA smears prepared from 1X TE buffer.
4 The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.

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

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications. Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

## Additional Material Required for Analysis with Fragment Analyzer Systems (not supplied)

| Instrument | Compatible Arrays | Part Number |
|---|---|---|
| 5200 Fragment Analyzer | FA 12 Capillary Array Ultrashort<br>FA 12 Capillary Array Short<br>FA 12 Capillary Array Long | A2300-1250-2247<br>A2300-1250-3355<br>A2300-1250-5580 |
| 5300 Fragment Analyzer. | FA 48 Capillary Array Short<br>FA/ZAG 96 Capillary Array Short<br>FA/ZAG 96 Capillary Array Long | A2300-4850-3355<br>A2300-9650-3355<br>A2300-9650-5580 |
| 5400 Fragment Analyzer | FA/ZAG 96 Capillary Array Short<br>FA/ZAG 96 Capillary Array Long | A2300-9650-3355<br>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 samples plates (*Refer to Appendix in Fragment Analyzer User Manual*)
- Multichannel pipettor and/or liquid handling device capable of dispensing 1-100 µL (sample plates) and 1,000 µL (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge
- 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


**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; vortex 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)<br>• Keep reagents at room temperature during sample preparation |
| Steps before sample preparation | • Allow reagents to equilibrate at room temperature for 30 min prior to use |
| Pipetting practice | • Pipette reagents against the side of the 96-well sample plate or sample tube<br>• Ensure no sample or Diluent Marker remains within or on the outside of the tip |
| 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 phenomena 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 application.<br>• 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.<br>• 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.<br>• For dsDNA fragments a target concentration of 500-600 pg/µL is recommended to provide maximum sizing accuracy. |
| Mixing and centrifugation recommendations | • Apply a new seal to 96-well sample plate prior to mixing and centrifugation<br>• When mixing sample with Diluent Marker (DM), mix the contents of the well thoroughly**.** It is suggested to perform ***one*** of the following methods to ensure complete mixing:<br>· 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. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells.<br>· 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.<br>· Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample or ladder volume.<br>• 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.<br>• Input range of concentration should not exceed 20,000 RFU |

## 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 (µL) | 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 |

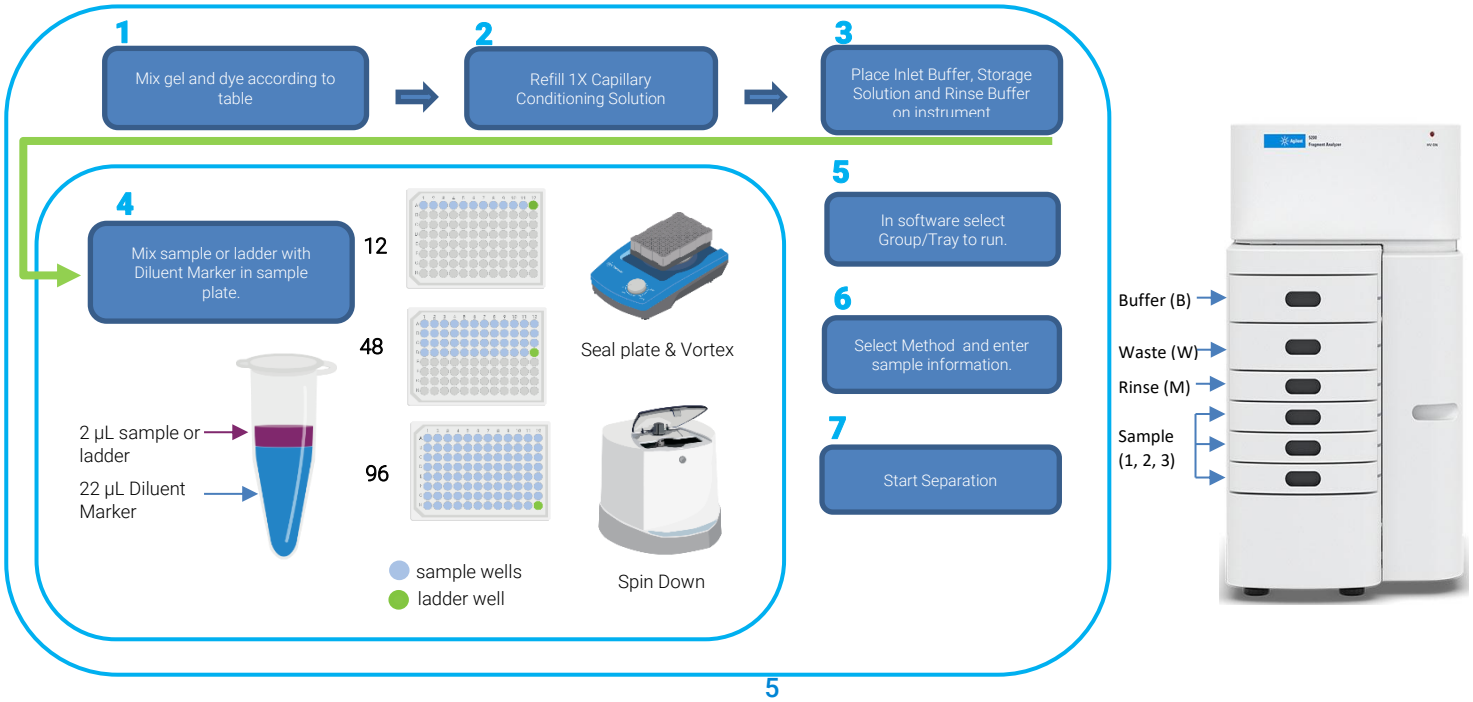
## Agilent HS Large Fragment DNF-464 Kit Operating Procedure

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

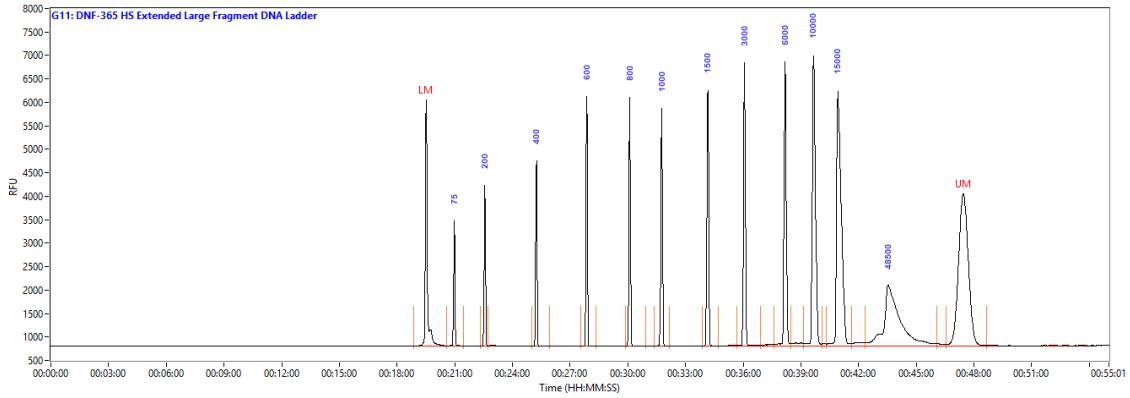
   Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.
   - 5200 – row H, 1 mL/well, drawer B
   - 5300 – 48 capillary, rows A-D, 100 µL/well, drawer 3
   - 5300/5400 – 96 capillary, all rows, 100 µL/well, drawer 3

   Place 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M".
   - 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, 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 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.<br>2 Verify sample was correctly added and mixed to sample well. |
| | 2 Sample not added to Diluent Marker solution or not mixed 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 using a 96-deepwell plate. 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 broad, signals are lower than expected, and/or migration time longer than expected. | 1 Capillary array needs to be reconditioned.<br>2 Capillary array vent valve is clogged. | 1 Flush array with 0.5 N NaOH solution and repeat experiment.<br>2 Clean vent valve with deionized water. (See User Manual for details). |
| 48,500 bp 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. |

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Not for use in Diagnostic Procedures.

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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