# Agilent DNF-468 HS Genomic DNA 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-468 HS Genomic DNA 50 kb Kit is designed for assessing the integrity, approximate size and quantitation of genomic DNA at low sample concentrations.

## Specifications

| Analytical Specifications[1,2] | HS Genomic DNA 50 kb Kit |
|---|---|
| Sizing Range | 75 bp – 60,000 bp |
| gDNA Concentration Range[1] | 0.3 ng/µL – 12 ng/µL input gDNA |
| gDNA Quantification Precision[1] | 25% CV |
| gDNA Quantification Accuracy[1] | ± 30% |
| Maximum gDNA Concentration | 12 ng/µL |
| **Physical Specifications** | |
| Total Electrophoresis Run Time | 22cm[2]: 50 minutes, 33cm: 60 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 human blood genomic DNA sample prepared in 1X TE buffer.

2 The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

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 |
|---|---|---|---|
| 5191-6570* | | HS Genomic DNA 50 kb, 500, 4°C | |
| | DNF-270-0240 | Genomic DNA 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-364-U125 | HS Extended Genomic DNA Ladder, 125 µL | 1 |
| | DNF-497-0125 | 0.25x TE Rinse Buffer, 125 mL | 1 |
| DNF-468-FR* | | HS Genomic DNA 50 kb, FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 1 |
| | DNF-375-0003 | HS Genomic DNA Diluent Marker, 2.4 mL | 5 |
| 5191-6612* | | Quantitative DNA, RT | 1 |
| | DNF-475-0050 | 5x Capillary Conditioning Solution, 50 mL | 1 |
| | C275-130 | Eppendorf LoBind 0.5 mL tubes (bag of 50) | 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 | A2300-1250-2247<br>A2300-1250-3355 |
| 5300 Fragment Analyzer. | FA 48 Capillary Array Short<br>FA/ZAG 96 Capillary Array Short | A2300-4850-3355<br>A2300-9650-3355 |
| 5400 Fragment Analyzer | FA/ZAG 96 Capillary Array Short | A2300-9650-3355 |

### 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 µL (sample plates) and 1,000 µL (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

| | |
|---|---|
| Environmental conditions | • Ambient operating temperature: 19 – 25 °C (66 – 77 °F)<br>• Keep insturment reagents at room temperature during sample preparation. |
| Sample Input Concentration | • Ensure sample input concentrations lie within kit specifications.<br>• Sample signal should not exceed 60,000 RFU. |
| Steps before sample preparation | • Allow instrument reagents to equilibrate at room temperature for 30 min prior to use<br>• Before sampling, sample gDNA must be equilibrated at room temperature for at least 30 min.<br>• Mix gDNA samples by vortexing or pipetting up-down before sampling to ensure a more homogeneous sample. |
| Pipetting practice | • Pipette reagents against the side of the 96-well sample plate or sample tube<br>• Ensure no sample or Diluent Marker (DM) remains within or on the outside of the tip |

| | |
|---|---|
| Ladder Preparation | • **The Lambda DNA fragment (48,500 bp) in the HS Extended Genomic DNA Ladder is sensitive to degradation.** The ladder should be stored at 2-8°C.<br>• Do not manually mix the HS Extended Genomic DNA Ladder by repeated inverting of the tube or repeated pipetting up/down, as this will result in the degradation of the Lambda DNA fragment in the ladder. The ladder solution can only be vortexed by a vortex mixer<br>• Before use, aliquot the HS Genomic Ladder into 10 µL aliquots (12 tubes) using the Eppendorf LoBind 0.5 mL tubes provided. Store at 2-8°C<br>• Do not pipette the ladder aliquots up and down nor flick the tube to mix as this may induce degradation of the Lamda DNA ladder fragment<br>• Gently vortex the aliquot and spin the ladder tube prior to use. Each aliquot is good for 4 uses<br>• The HS Genomic Ladder should be run in parallel with the samples for each experiment. To prepare the working HS Extended Genomic DNA Ladder solution:<br>a) Add 22 µL of DM solution into an Eppendorf LoBind 0.5 mL tube (supplied with kit)<br>b) Add 2 µL of the HS Extended Genomic DNA Ladder<br>c) Gently vortex the tube. Mix the working HS Extended Genomic DNA Ladder solution only by vortexing.<br>• Pipette the entire 24 µL of the working HS Extended Genomic DNA Ladder in the designated ladder well. |
| Sample Plate Preparation | • Pipette 22 µL of DM solution to each sample well in a row that is to contain sample<br>• Do not add any DM solution to the well reserved for the working Ladder solution<br>• Fill any unused wells within the row of the sample plate with 24 µL of blank solution.<br>• Pipette 2 µL of each genomic DNA sample into the 22 µL of DM solution in the respective wells of the sample plate.<br>• Mix contents of the well:<br>  • using a separate pipette tip with the pipettor set to ~20 µL voluime, pipette each well up/down about 10 times to further mix.<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.<br>• After mixing, centrifuge the plate to remove any air bubbles. Centrifugation should be done at a speed low enough to remove air bubbles as well as avoid settling of genomic DNA at the bottom of the sample well. High speed centrifugation can cause genomic DNA to settle at the bottom of the sample wells, leading to sampling errors and less accurate quantification. **A recommended relative centrifugal force (RCF) limit is 100 x g for less than 30 seconds.**<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. |

## 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 |

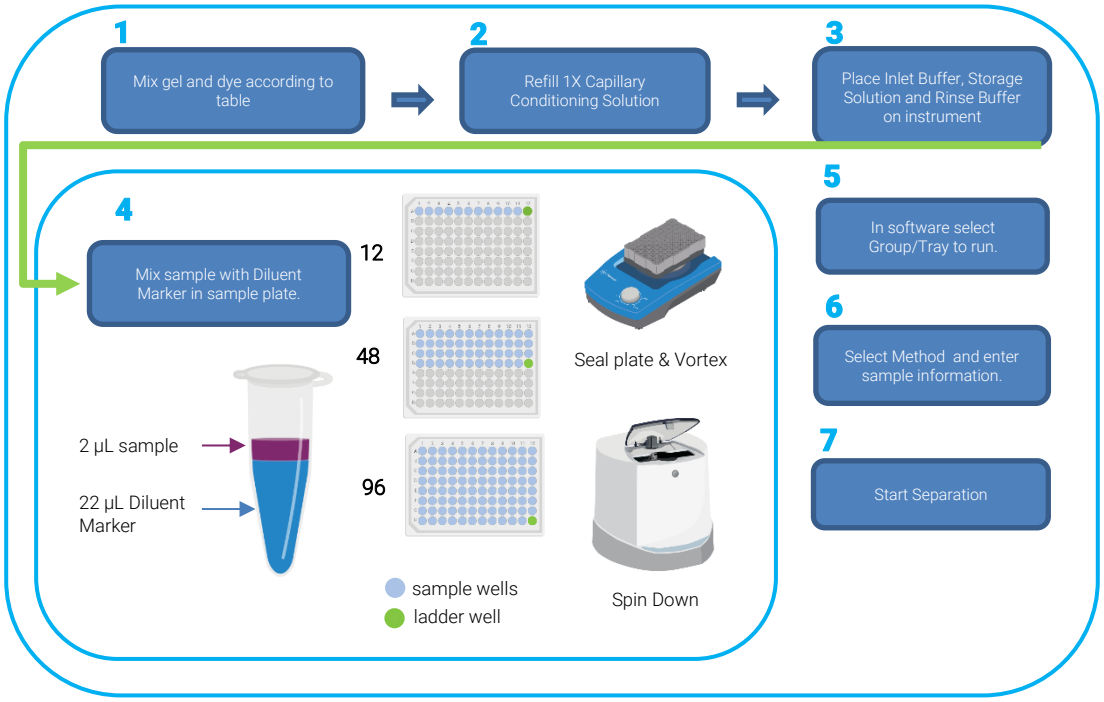
## Agilent HS Genomic DNA 50 kb DNF-468 Kit Operating Procedure

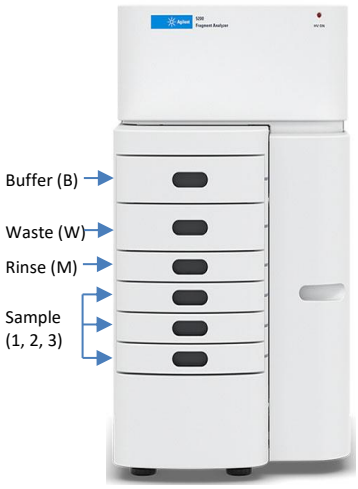
1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1X Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer 'W".
4. Place 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 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

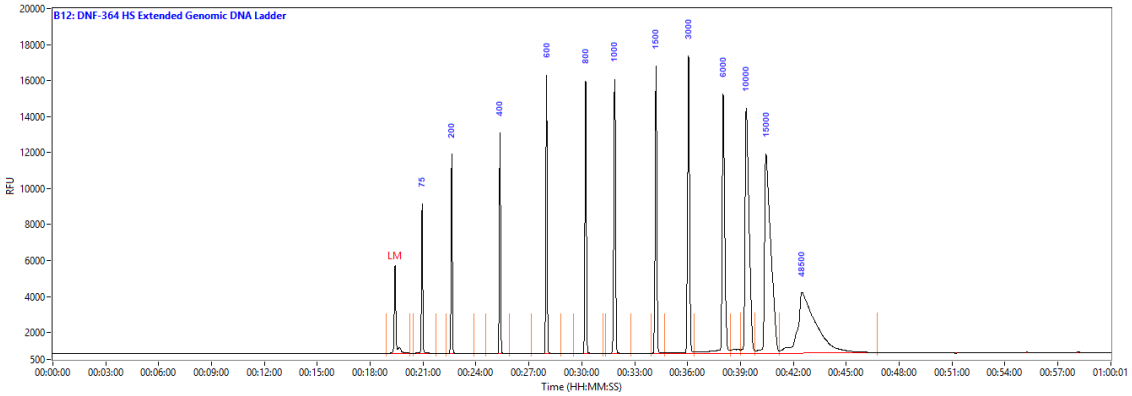
   Place 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M". Replace daily.
   - 5200 – Row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows
5. Mix samples with diluent marker in sample plate, add 24 uL of Blank Solution to unused wells. Place ladder in corresponding well (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-468-22
   - DNF-468-33

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



## HS Genomic DNA 50 kb Ladder result




Representative HS Genomic DNA Ladder result using the Fragment Analyzer systems with the DNF-468 HS Genomic DNA 50 kb kit. The peaks are annotated by size (bp). Method: DNF-468-33 (short capillary 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-468 HS Genomic DNA 50 kb kit and suggested remedies. Contact Agilent Technical Support if you have any additional troubleshooting or maintenance questions.

| Issue | Cause | Corrective Action |
|---|---|---|
| No peak observed for sample when expected. Lower marker peak observed. | 1 Sample concentration too low and out of range. | 1 Prepare more concentrated sample and repeat experiment. |
| | 2 Sample not homogenously mixed before sampling. | 2 Make sure the sample is equilibrated to room temperature for at least 30 min before use, vortex the sample or pipette up-down to mix before sampling. |
| | 3 Sample highly degraded; no dye intercalates. | 3 Sample not suitable for use. |
| Much lower concentration obtained for gDNA sample than expected. | 1 Sample contains very high molecular weight (HMW), aggregated genomic DNA (>>60 kb). | 1 The analysis of HMW, aggregated genomic DNA can result in lower than expected concentration values due to the nature of sample aggregation, which can inhibit sample injection. Analysis of these types of samples at lower concentrations may improve the quantitation. |
| Extra peaks/smear near lower marker observed (10-1000bp). | 1 Genomic DNA possibly contaminated with RNA. | 1 Remove RNA contaminants from the genomic DNA sample and reanalyze; OR exclude the extra peaks in data processing for better quantitation and sizing accuracy. |
| Degradation of the 48,500 bp Lambda DNA fragment in the ladder. | 1 Ladder solution was manually mixed by repeated inverting of the tube or repeated pipetting up/down, or ladder solution was exposed to freeze/thaw cycles. | 1 Prepare fresh ladder solution. Ensure ladder is stored at 2-8°C. Mix the ladder solution only by vortexing and avoid freeze/thaw cycles. |

| | | |
|---|---|---|
| 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.<br>2 Insufficient sample volume. A minimum of 20 µL is required.<br>3 Capillary is plugged. | 1 Check sample plate wells for trapped air bubbles. Centrifuge plate.<br>2 Verify proper volume of solution was added to sample well<br>3 Check waste plate for liquid in the capillary well by using a 96-deepwell plate. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array. |

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