

# Agilent DNF-467 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-467 Genomic DNA 50 kb Kit is designed for assessing the integrity, approximate size and quantitation of genomic DNA.

### Specifications

Analytical Specifications <sup>1,2</sup>	Genomic DNA 50 kb Kit
Sizing Range	75 bp – 60,000 bp
gDNA Concentration Range <sup>2</sup>	25 ng/μL – 250 ng/μL input DNA
gDNA Quantification Precision <sup>2</sup>	25% CV
gDNA Quantification Accuracy <sup>2</sup>	± 30%
Maximum gDNA Concentration	250 ng/μL
Physical Specifications	
Total Electrophoresis Run Time	22cm <sup>1</sup> : 50 minutes, 33cm: 60 minutes
Samples Per Run	12, 48 or 96; depending on the instrument type
Sample Volume Required	1 μL
Guaranteed Shelf Life	4 months

<sup>1</sup> The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

<sup>2</sup> Results using human blood genomic DNA in 1X TE buffer.

**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
5191-6569*		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-367-U050	Extended Genomic DNA Ladder, 50 µL	1
	DNF-375-0120	HS Genomic DNA Diluent Marker, 120 mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
5191-6612*		Quantitative DNA, RT	
	DNF-475-0050	5x Capillary Conditioning Solution, 50 mL	1
	C275-130	Eppendorf LoBind 0.5 mL tubes (bag of 50)	1
	DNF-600-U030	Intercalating Dye, 30 µL	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 FA 12 Capillary Array Short	A2300-1250-2247 A2300-1250-3355
5300 Fragment Analyzer.	FA 48 Capillary Array Short FA/ZAG 96 Capillary Array Short	A2300-4850-3355 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	<ul style="list-style-type: none"> <li>Ambient operating temperature: 19 – 25 °C (66 – 77 °F)</li> <li>Keep instrument reagents at room temperature during sample preparation.</li> </ul>
Sample Input Concentration	<ul style="list-style-type: none"> <li>Ensure sample input concentrations lie within kit specifications.</li> <li>Sample signal should not exceed 60,000 RFU.</li> </ul>
Steps before sample preparation	<ul style="list-style-type: none"> <li>Allow reagents to equilibrate at room temperature for 30 min prior to use.</li> <li>Before sampling, sample gDNA must be equilibrated at room temperature for at least 30 min.</li> <li>Mix gDNA samples by vortexing or pipetting up-down before sampling to ensure a more homogeneous sample.</li> </ul>
Pipetting practice	<ul style="list-style-type: none"> <li>Pipette reagents against the side of the 96-well sample plate or sample tube.</li> <li>Ensure no sample or Diluent Marker remains within or on the outside of the tip.</li> </ul>

**Ladder Preparation**

- **The Lambda DNA fragment (48,500 bp) in the Extended Genomic DNA Ladder is sensitive to degradation.** The ladder should be stored at 2-8°C.
- Do not manually mix the 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
- The Extended Genomic DNA Ladder should be run in parallel with the samples for each experiment. To prepare the working Extended Genomic DNA Ladder Solution:
  - a) Add 199  $\mu$ L of diluent marker (DM) into a separate 0.5 mL Eppendorf DNA LoBind tube (supplied with kit)
  - b) Gently vortex the vial containing the Extended Genomic DNA Ladder, then pipette 1  $\mu$ L of the Extended Genomic DNA Ladder into the 199  $\mu$ L of the DM. Mix the working Genomic DNA Ladder solution only by vortexing.
- Pipette the entire 200  $\mu$ L of the working Extended Genomic DNA Ladder solution into the designated ladder well

**Sample Plate Preparation**

- Pipette 199  $\mu$ L of HS Genomic DNA Diluent Marker (DM) to each well in a row of the plate that is to contain sample
- Do not add any DM to the well reserved for the Extended Genomic DNA Ladder
- Fill any unused wells within the row of the sample plate with 24  $\mu$ L of Blank Solution
- Pipette 1  $\mu$ L of each genomic DNA sample into the 199  $\mu$ L of DM in the respective wells of the sample plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip
- When mixing sample with DM it is important to mix the contents of the wells thoroughly to achieve the most accurate quantification. After adding 1  $\mu$ L of sample to the 199  $\mu$ L of DM, use a separate pipette tip with the pipettor set to  $\sim$ 100  $\mu$ L volume, and pipette each well up/down about 10 times to further mix.
- 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.**
- 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 the risk of leaking. Ensure the gel + dye is mixed without generating bubbles, gently invert tube 5-10 times.


Number of Samples	Intercalating Dye Volume ( $\mu$ 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

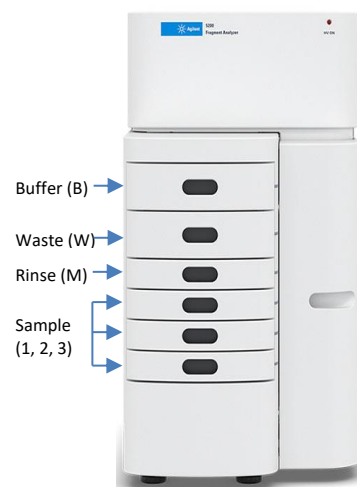
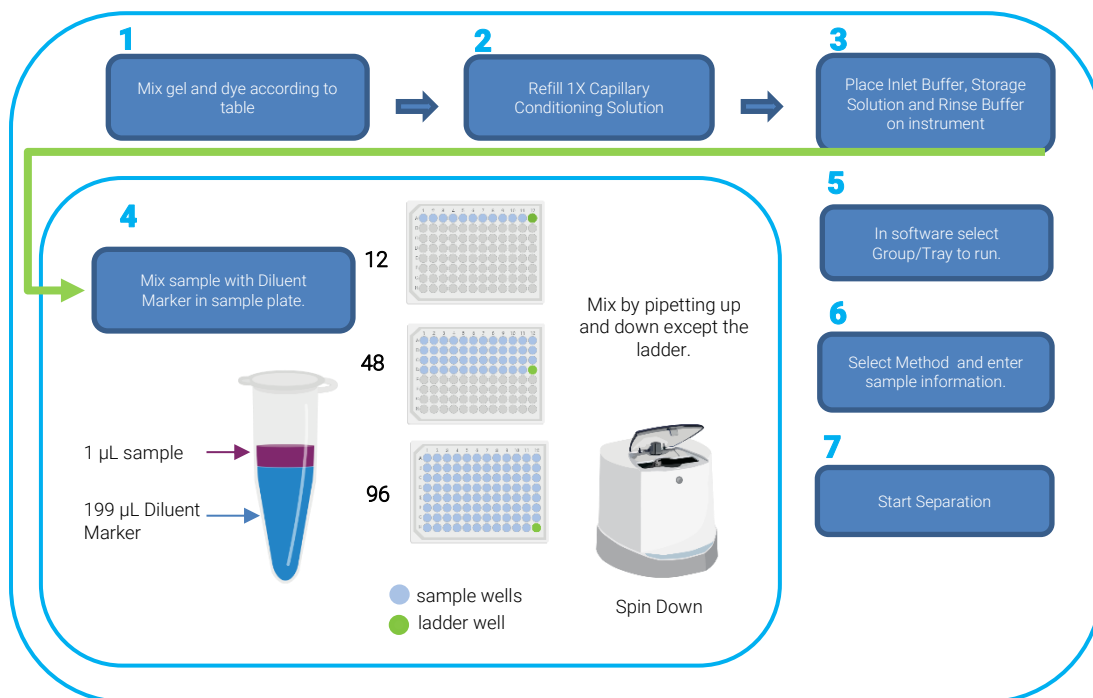
**Conditioning Solution**

The provided 5X Conditioning Solution must be diluted to 1X using submicron DI water prior to use. Invert to mix.

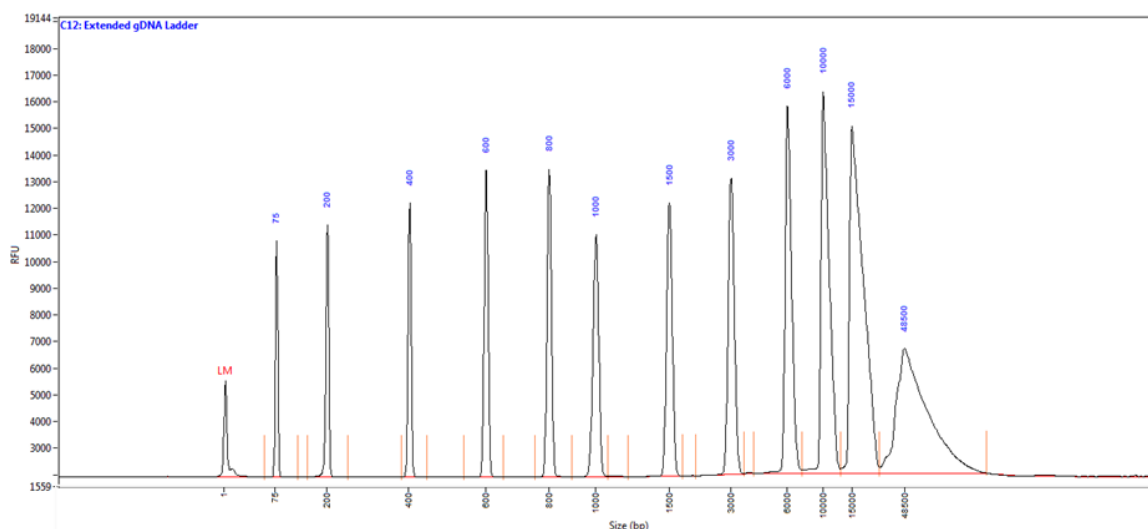
Number of Samples	Volume of 1X Conditioning Solution (mL)
12	10
24	15
48	25
96	45
192	80
384	160

## Agilent Genomic DNA 50 kb DNF-467 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  $\mu$ L/well, drawer 3
  - 5300/5400 – 96 capillary, all rows, 100  $\mu$ L/well, drawer 3
 Place 0.25x TE Rinse Buffer plate, 200  $\mu$ L/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  $\mu$ L 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-467-22
  - DNF-467-33
 Enter Tray Name, Folder Prefix and Notes, if desired.
8. Add method to the queue by selecting "OK", press play  to start the separation.



## Extended Genomic DNA Ladder result



Representative Extended Genomic DNA Ladder result using the Fragment Analyzer systems with the DNF-467 Genomic DNA 50 kb kit. The peaks are annotated by size (bp). Method: DNF-467-33 (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-467 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. 2 Sample not homogenously mixed before sampling. 3 Sample not added to DM. 4 Sample highly degraded; no dye intercalates.	1 Prepare more concentrated sample and repeat experiment; OR prepare sample and analyze with DNF-468 HS Genomic DNA 50 kb kit. 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 Verify sample was correctly added. 4 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 with this kit or with the DNF-468 HS Genomic DNA 50 kb kit may improve the quantitation.
Extra peaks/smear near lower marker observed (10-1000 bp).	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 4C. Mix the ladder solution only by-vortexing and avoid freeze/thaw cycles.

No sample peak or marker peak observed for individual sample.	<ol style="list-style-type: none"> <li>1 Air trapped at the bottom of the sample plate well, or bubbles present in sample well.</li> <li>2 Insufficient sample volume. A minimum of 20 <math>\mu</math>L is required.</li> <li>3 Capillary is plugged.</li> </ol>	<ol style="list-style-type: none"> <li>1 Check sample plate wells for trapped air bubbles. Centrifuge plate.</li> <li>2 Verify proper volume of solution was added to sample well.</li> <li>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.</li> </ol>
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### Technical Support and Further Information

For technical support please visit [www.agilent.com](http://www.agilent.com) which offers useful information and support regarding the products and technology.

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SD-AT000128 Edition 03/25

