Agilent DNF-473 NGS Fragment 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-473 NGS Fragment kit is designed for the quantitative and qualitative analysis of NGS libraries and their intermediates from 100 to 6,000 bp.

Specifications

Analytical Specifications[1,2,3,4]	NGS Fragment Kit
Sizing Range	100 bp – 6,000 bp
Separation Resolution	100 bp – 1,000 bp ≤ 5%; 1000 bp – 6,000 bp ≤ 10% (short capillary array, 33cm)
Sizing Accuracy[2,3]	± 5% or better
Sizing Precision[2,3]	2% CV
Fragment Concentration Range[2]	0.1 ng/µL – 10 ng/µL input DNA
Smear Concentration Range[4]	5 ng/µL – 100 ng/µL input DNA
Quantification Accuracy[2,4]	± 25%
Quantification Precision[2,4]	15% CV
Maximum Concentration	10 ng/µL per fragment; 100 ng/µL per total sample
Physical Specifications	
Total Electrophoresis Run Time	22cm[1]: 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
Guaranteed Shelf Life	4 months

1 The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.
2 Results using 300 bp and 1000 bp DNA fragment standards in 1X TE buffer.
3 Results using DNA ladder in 1X TE buffer.
4 Results using sheared gDNA with smear range 50 bp - 2000 bp 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
5191-6576*		NGS Fragment (1-6000 bp), 500, 4°C	
	DNF-240-0240	NGS Separation Gel, 240 mL	1
	DNF-302-0008	BF-2000 Blank Solution, 8 mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-473-FR*		NGS Fragment (1-6000 bp), FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-374-0003	NGS Diluent Marker (1-6000 bp), 2.4 mL	5
	DNF-399-U100	NGS DNA Ladder, 100 µL	1
DNF-475-0050	DNF-475-0050	5x Capillary Conditioning Solution, 50 mL	1

Kit Components – 1000 Sample Kit - *Refer to product label for proper storage conditions*

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6577*		NGS Fragment (1-6000 bp), 1000, 4°C	
	DNF-240-0500	NGS Separation Gel, 500 mL	1
	DNF-302-0008	BF-2000 Blank Solution, 8 mL	1
	DNF-355-0300	5x 930 dsDNA Inlet Buffer, 300 mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-473-FR*		NGS Fragment (1-6000 bp), FR	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	DNF-374-0003	NGS Diluent Marker (1-6000 bp), 2.4 mL	10
	DNF-399-U100	NGS DNA Ladder, 100 µL	2
DNF-475-0100	DNF-475-0100	5x Capillary Conditioning Solution, 100 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 FA 12 Capillary Array Short FA 12 Capillary Array Long	A2300-1250-2247 A2300-1250-3355 A2300-1250-5580
5300 Fragment Analyzer.	FA 48 Capillary Array Short FA/ZAG 96 Capillary Array Short FA/ZAG 96 Capillary Array Long	A2300-4850-3355 A2300-9650-3355 A2300-9650-5580
5400 Fragment Analyzer	FA/ZAG 96 Capillary Array Short FA/ZAG 96 Capillary Array Long	A2300-9650-3355 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 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

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) • 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.
Steps before sample preparation	• Allow instrument 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 • Ensure no sample or Diluent Marker remains within or on the outside of the tip
Mixing and centrifugation recommendations	• 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: · 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. · 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. • Fill any unused wells within the row of the sample plate with 24 µL Blank Solution. • After mixing, centrifuge the plate to remove any air bubbles. • 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

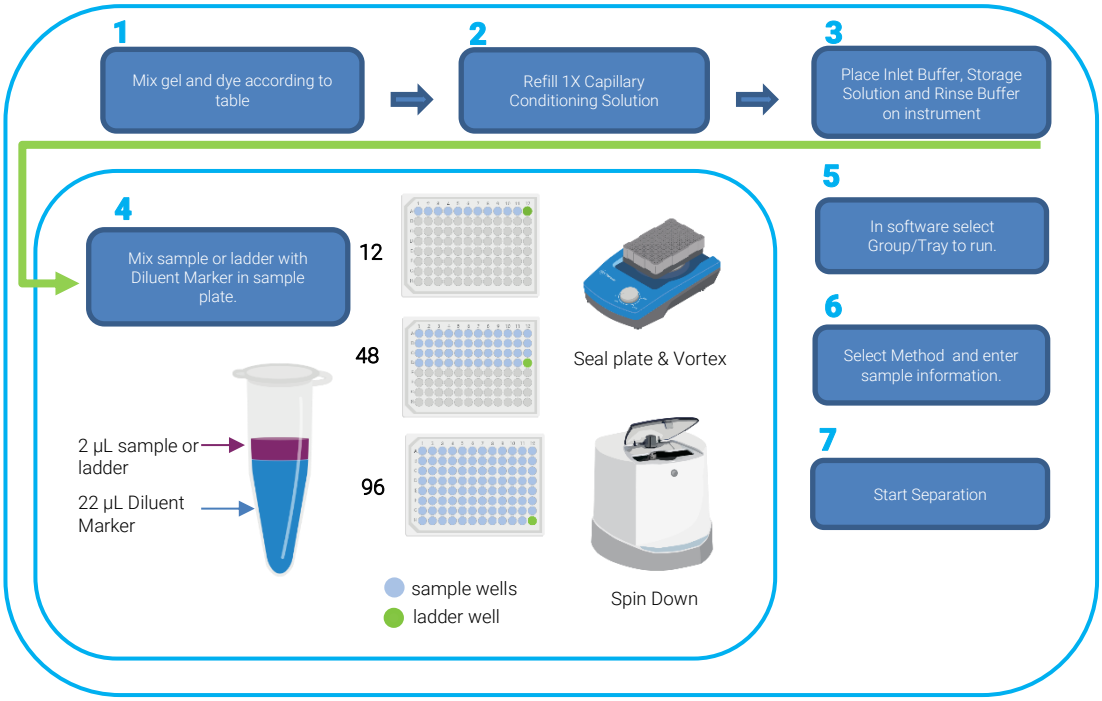
Agilent NGS Fragment DNF-473 Kit Operating Procedure

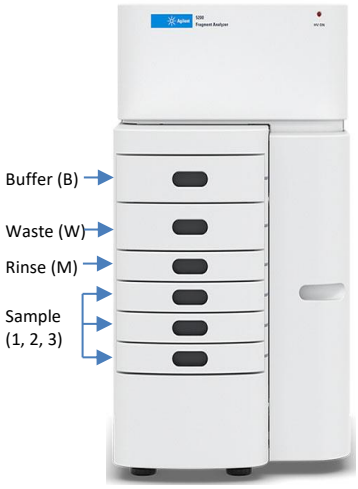
1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1X Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer 'W".
4. Place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

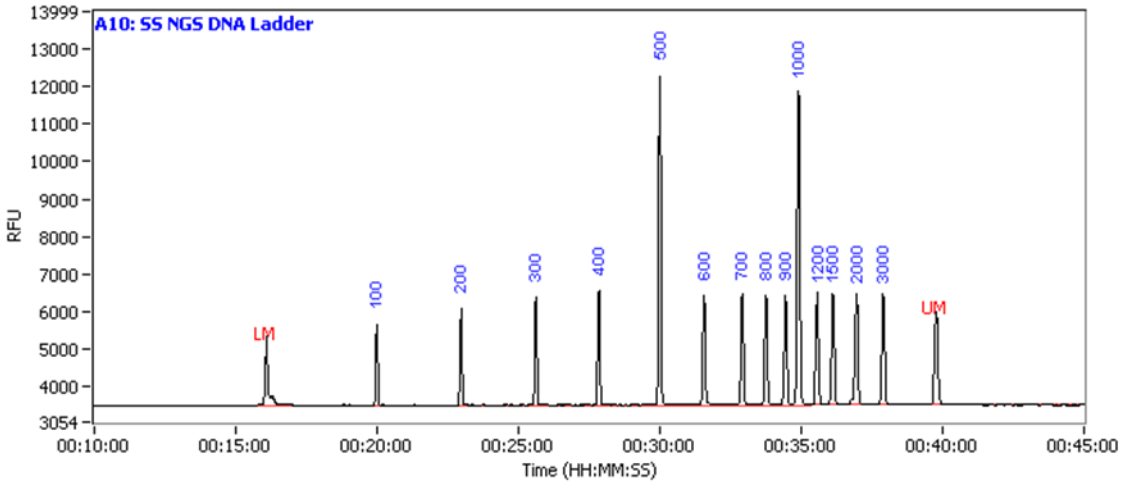
   Place 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M". Replace daily.
   - 5200 – Row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows
5. Mix samples or ladder with diluent marker in sample plate, add 24 µL of Blank Solution to unused wells. Place ladder in corresponding 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-473-22
   - DNF-473-33
   - DNF-473-55

   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



Representative NGS DNA Ladder result using the Fragment Analyzer system with the DNF-473 HS NGS Fragment kit (1bp – 6,000bp). Method: DNF-473-33 (short array). Peaks annotated by size (bp). RFU values may differ between instruments.

Troubleshooting

The following table lists several potential kit specific issues which may be encountered when using the NGS Fragment kit (1-6000 bp) (Part #DNF-473) and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
The peak signal is >> 60,000 RFU; upper marker peak is low or not detected relative to lower marker.	1 Input DNA sample concentration is too high. Ensure total signal height does not exceed 2,000 RFU (smear) or 60,000 RFU (fragment), or total input DNA concentration does not exceed recommended limits.	1 Dilute input DNA sample concentration with 1X TE buffer and repeat experiment; OR
DNA Sample smear overlaps with Lower/Upper Marker peak.	1 Input DNA sample size distribution outside of assay range. 2 Input DNA sample concentration too high.	1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment; OR Prepare fresh sample using Large Fragment Kit (part #DNF-492) 2 Dilute input DNA sample concentration with 1X TE buffer and repeat experiment.
No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1 Sample concentration too low and out of range. 2 Sample not added to Diluent Marker solution or not mixed well.	1 Prepare more concentrated sample and repeat experiment OR Prepare fresh sample and analyze with HS NGS Fragment Kit (part #DNF-474) 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 the sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 µL is required. 3 Capillary is plugged.	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.

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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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