Agilent DNF-477 HS Small Fragment Kit Quick Guide for the Fragment Analyzer Systems

The Agilent Fragment Analyzer systems are automated capillary electrophoresis platforms for scalable, flexible, fast, and reliable electrophoresis of nucleic acids.

This Quick Guide is intended for use with the Agilent 5200, 5300, and 5400 Fragment Analyzer systems only. The HS Small Fragment kit (p/n DNF-477-0500) is designed for sizing and quantification of dsDNA smears/fragments between 50 bp and 1,500 bp. Example applications include quality control of Next Generation Sequencing (NGS) libraries or quantitative PCR fragment analysis.

Specifications

Analytical specifications	HS Small Fragment assay
Sizing Range	50 bp – 1,500 bp
Sizing Accuracy[1]	± 5% or better
Sizing Precision[1]	2% CV
Separation Resolution[1]	50 bp –700 bp ≤ 5%; 700 bp –1,500 bp ≤ 10% (ultrashort capillary array, 22 cm)[3] 50 bp –900 bp ≤ 5%; 900 bp – 1,500 bp ≤ 10% (short capillary array, 33 cm)
Fragment Concentration Range[1]	5 pg/µL - 500 pg/µL input DNA
Smear Concentration Range[2]	100 pg/µL – 5,000 pg/µL input DNA
Quantification Accuracy[1]	± 25%
Quantification Precision[1]	15% CV
Maximum Concentration	500 pg/µL per fragment; 5,000 pg/µL per total sample
Physical Specifications	
Total electrophoresis run time	22cm[1]: 33 minutes, 33cm: 40 minutes, 55cm: 75 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 500 bp DNA fragment standards and DNA Ladder in 1x TE buffer.

2 Results using sheared gDNA with smear range from 10 bp – 1,400 bp in 1x TE buffer with 33-55 Array.

3 The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.

Kit Components – 500 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6581*		HS Small Fragment, 500, 4°C	
	DNF-230-0240	Small Fragment Separation Gel, 240 mL	1
	DNF-300-0008	BF-25 Blank Solution, 8mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL • Dilute with sub-micron filtered water prior to use	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
DNF-477-FR*		HS Small Fragment, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	DNF-371-0003	HS Small Fragment Diluent Marker, 2.4 mL	5
	DNF-372-U100	HS Small Fragment DNA Ladder, 100 µL	1
DNF-475-0050	DNF-475-0050	5x Capillary Conditioning Soln, RT • Dilute with sub-micron filtered water prior to use	1

*Not orderable.

WARNING

- 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 goggles, safety gloves and protective clothing.

Additional Material Required for Analysis with the Fragment Analyzer Systems

- Fragment Analyzer systems with LED fluorescence detection:
- 5200 Fragment Analyzer system (p/n M5310AA)
    - FA 12-Capillary Array Ultrashort, 22 cm (p/n A2300-1250-2247) OR
    - FA 12-Capillary Array Short, 33 cm (p/n A2300-1250-3355) OR
    - FA 12-Capillary Array Long, 55 cm (p/n A2300-1250-5580)
- 5300 Fragment Analyzer system (p/n M5311AA)
    - FA 48-Capillary Array Short, 33 cm (p/n A2300-4850-3355) OR
    - FA/ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
    - FA/ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580)
- 5400 Fragment Analyzer system (p/n M5312AA)
    - FA 48-Capillary Array Short, 33 cm (p/n A2300-4850-3355) OR
    - FA/ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
    - FA/ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580):
- Agilent Fragment Analyzer controller software (Version 1.1.0.11 or higher)
- Agilent ProSize data analysis software (Version 2.0.0.61 or higher)

Additional equipment/reagents required (not supplied)

- 96-well PCR sample plates. Please refer to Appendix – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer System User Manual for a complete approved sample plate list
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 µL volumes (sample plates) and 1,000 µL volumes (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 5x 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solution)
- 96-deepwell 1mL plate: Fisher Scientific #12-566-120 (inlet buffer and/or waste plate)
- Reagent reservoir, 50 mL (VWR #89094-680 or similar) (for use in pipetting inlet buffer plates/sample trays)
- Conical centrifuge tubes for prepared separation gel/dye mixture and/or 1x Capillary Conditioning Solution
    - 50 mL (for 5200 Fragment Analyzer system or 50 mL volumes): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
- 250 mL (for 5300 and 5400 Fragment Analyzer systems or larger volumes): Corning #430776, available from Fisher Scientific #05-538-53 or VWR #21008-771
- Vortexer (for mixing of samples, ladders, and/or markers in tubes and/or plates)
- Capillary Storage Solution (p/n GP-440-0100)

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
Steps before sample preparation	• Allow reagents to equilibrate at room temperature for 30 min prior to use
Pipetting practice	• Pipette reagents carefully against the side of the 96-well sample plate or sample tube • Ensure that no sample or Diluent Marker remains within or on the outside of the tip

Mixing and centrifugation recommendations	• Apply a new seal to 96-well sample plate prior to mixing and centrifugation
	• When mixing sample with Diluent Marker (DM), it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. It is highly suggested to perform one of the following methods to ensure complete mixing. After mixing, briefly centrifuge and visually confirm that all liquid is collected at the bottom of the 96-well sample plate or tube strips and any air bubble is removed
	  ◦ 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. 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.
	  ◦ 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. Some models enable using the pipette tip for both adding and mixing.
	• 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

Prepare gel/dye mixture for 5200, 5300, and 5400 Fragment Analyzer Systems. To ensure the gel/dye mixture is mixed homogeneously without generating bubbles, gently invert the centrifuge tube 5 to 10 times, depending on the volume of the mixture. **NOTE**: Centrifuge dye prior to opening the vial to reduce risk of leaking.

5200 Fragment Analyzer system volume specifications

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
12	1.0 µL	10 mL	10 mL
24	1.5 µL	15 mL	15 mL
36	2.0 µL	20 mL	20 mL
48	2.5 µL	25 mL	25 mL
96	4.5 µL	45 mL	45 mL

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

5300 Fragment Analyzer system volume specifications with 48-capillary array

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
48	2.5 µL	25 mL	25 mL
96	4.0 µL	40 mL	40 mL
144	5.5 µL	55 mL	55 mL
192	7.0 µL	70 mL	70 mL
240	8.5 µL	85 mL	85 mL
288	10.0 µL	100 mL	100 mL

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

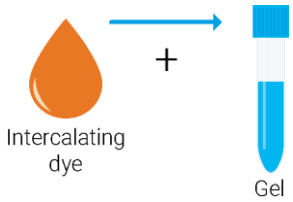
5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

Agilent HS Small Fragment DNF-477 assay operating procedure

1. Mix fresh gel and dye according to the volumes in the Gel preparation tables. Refill 1x Capillary Conditioning Solution as needed.



2. Place a fresh 1x 930 dsDNA Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.

   2.1. 5200 system; Fill row A of buffer plate

   2.2. 5300 system - 48 capillary; Fill rows A-D of buffer plate

   2.3. 5300/5400 system - 96 capillary; Fill all rows of buffer plate

3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

   3.1. 5200 system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B "

   3.2. 5300 system - 48 capillary; Fill rows A-D of a sample plate with 100 µL/well, place in drawer '3'

   3.3. 5300/5400 system - 96 capillary; Fill all rows of a sample plate with 100 µL/well, place in drawer '3'

      3.3.1. 5400 system; place in drawer "S"

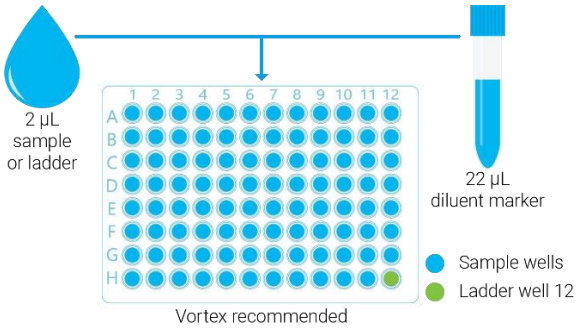
4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

   4.1. 5200 system; Fill row A of sample plate

   4.2. 5300 system - 48 capillary; Fill rows A-D of sample plate

   4.3. 5300/5400 system - 96 capillary; Fill all rows of sample plate

5. Mix samples or Ladder with Diluent Marker in sample plate, add 24 µL of BF-25 Blank Solution to unused wells. Place ladder in corresponding well dependent on the capillary size.



5200 system; Ladder – well 12, depending on which row is chosen

5300 system - 48 capillary; Ladder – well D12 or H12, depending on which group is chosen

5300/5400 system - 96 capillary; Ladder – well H12

WARNING

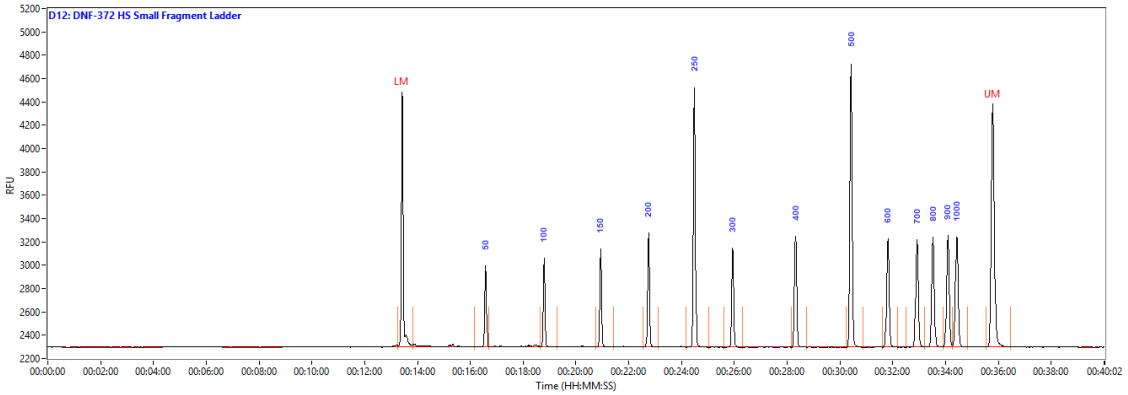
Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- Refer to product material safety datasheets for further chemical and biological safety information.
- Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Agilent Fragment Analyzer software operating procedure

1. Select Row, Group or Tray to run.
2. Enter **sample ID** and **Tray ID** (optional).
3. Select **Add to Queue**, from the dropdown menus select the corresponding method based on your capillary length;
   - 3.1 DNF-477-22 – HS Small Fragment
   - 3.2 DNF-477-33 – HS Small Fragment
   - 3.3 DNF-477-55 - HS Small Fragment
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

DNA Ladder result



Representative HS Small Fragment DNA Ladder result using the Fragment Analyzer system with the DNF-477 HS Small Fragment kit. The peaks are annotated by size (bp). Method: **DNF-477-33** (short array).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-477 HS Small Fragment kit and suggested remedies. Contact Agilent Technical Support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
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 for smear or 20,000 RFU for fragment, or total input DNA concentration does not exceed Recommended limits.	1 1 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment; **OR** Prepare fresh sample and analyze with Small Fragment kit (1bp – 1,500bp), (p/n DNF-476).
DNA sample smear overlaps with lower marker peak	1 Input DNA sample size distribution outside of assay range. 2 Input DNA sample concentration too high.	1 1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment. 2 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment.
DNA sample smear overlaps with upper marker peak.	1 Input DNA sample size distribution outside of assay range.	1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment; **OR** Prepare fresh sample and analyze with HS NGS Fragment kit (1 – 6,000bp), (p/n DNF-474); or HS Large Fragment 50kb kit (p/n DNF-464-0500).
No peak observed for DNA sample when expected. Lower/upper marker peaks observed.	1 1 Sample concentration too low and out of range. 2 Sample not added to DM solution or not mixed well.	1 Prepare more concentrated sample and repeat experiment. 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 20µL 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. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array.

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Not for use in Diagnostic Procedures.

Technical Support and Further Information

For technical support, please visit www.agilent.com. It offers useful information and support about the products and technology.

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