# Agilent DNF-476 Small 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-476 Small Fragment kit 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[1,2,3,4,5] | Small Fragment Kit |
|---|---|
| Sizing Range | 50 bp – 1,500 bp |
| Sizing Accuracy[3,4,5] | ± 5% or better |
| Sizing Precision[2,3] | 2% CV |
| Separation Resolution | 50 bp –700 bp ≤ 5%; 700 bp –1,500 bp ≤ 10% (ultrashort capillary array, 22 cm)[1]<br>50 bp –900 bp ≤ 5%; 900 bp – 1,500 bp ≤ 10% (short capillary array, 33 cm) |
| Fragment Concentration Range[3] | 0.1 ng/µL – 10 ng/µL input DNA |
| Smear Concentration Range[4] | 5 ng/µL – 100 ng/µL input DNA |
| Quantification Accuracy[3,4] | ± 25% |
| Quantification Precision[3,4] | 15% CV |
| Maximum Concentration | 10 ng/µL per fragment: 100 ng/µL per total sample |
| **Physical Specifications** | |
| Total Electrophoresis Run Time | 22cm[1]: 33 minutes, 33cm: 45 minutes, 55cm: 75 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 Specifications not applicable to the FA 12-Capillary Array Long, 55 cm and FA/ZAG 96-Capillary Array Long, 55 cm.
3 Results using 400 bp DNA fragment standard in 1X TE buffer.
4 Results using sheared gDNA with smear range from 10 bp- 1,400 bp in 1X TE buffer.
5 Results using DNA Ladder 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 |
|---|---|---|---|
| 5191-6580* | | Small Fragment, 500, 4 °C | |
| | DNF-230-0240 | Small 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-497-0125 | 0.25x TE Rinse Buffer, 125 mL | 1 |
| DNF-476-FR* | | Small Fragment, FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 1 |
| | DNF-362-0003 | Small Fragment Diluent Marker, 2.4 mL | 5 |
| | DNF-363-U100 | Small Fragment DNA Ladder, 100 µL | 1 |
| DNF-475-0050 | 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 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)<br>• Keep instrument 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 insturment 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 |
| **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:<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>• Fill any unused wells within the row of the sample plate with 24 µL of Blank Solution.<br>• After mixing, centrifuge the plate to remove any air bubbles.<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 |

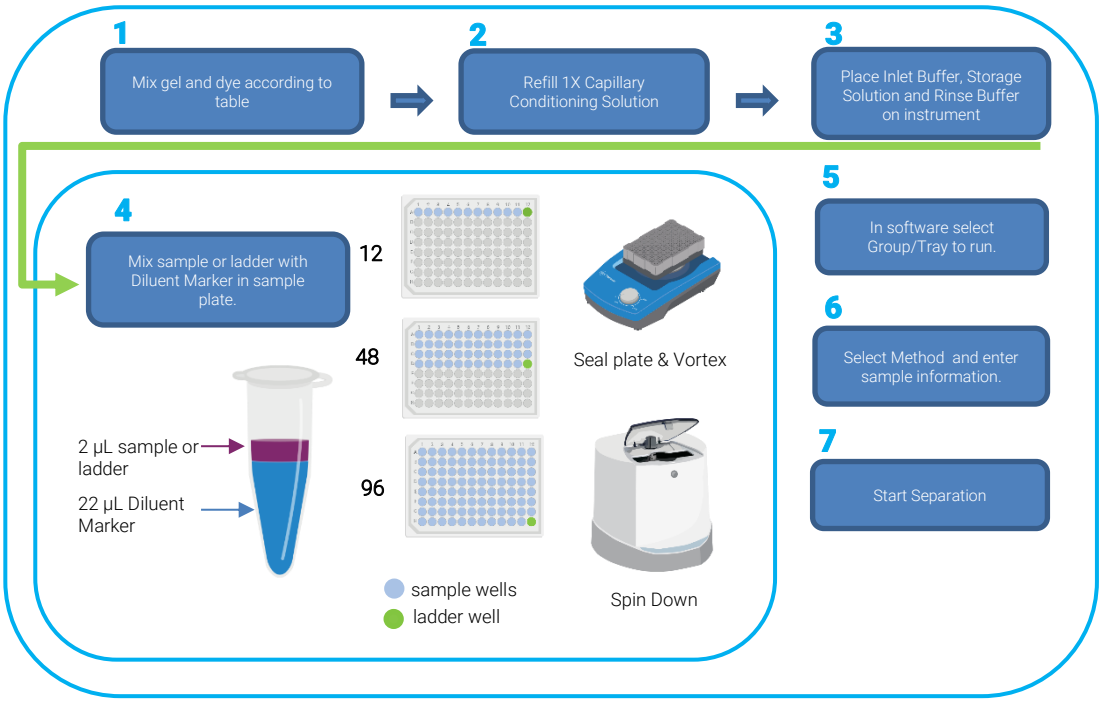
## Agilent Small Fragment DNF-476 Kit Operating Procedure

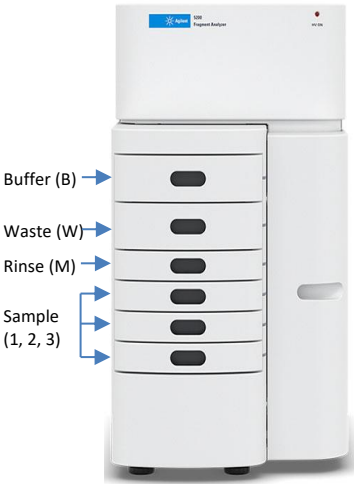
1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1X Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer 'W".
4. Place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

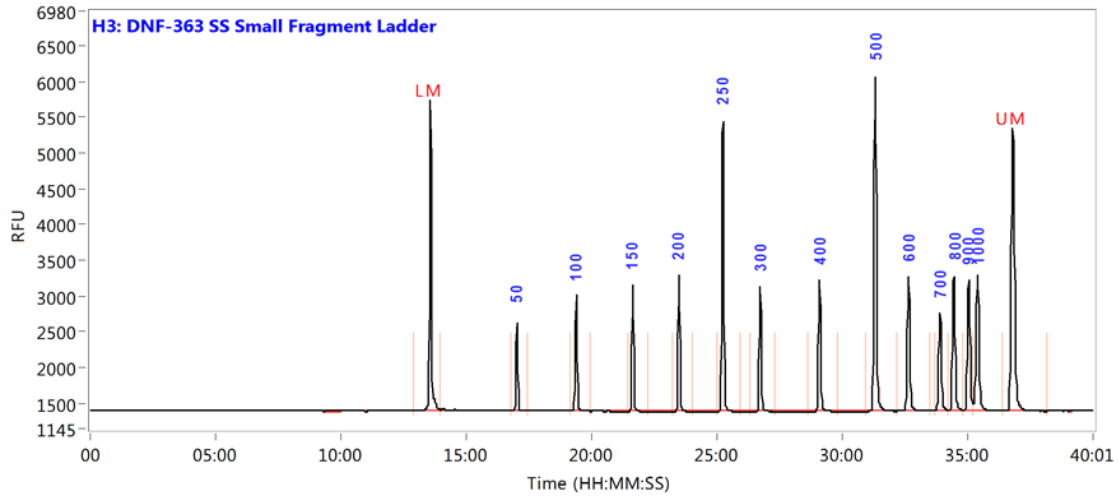
   Place 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M". Replace daily.
   - 5200 – Row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows
5. Mix samples or ladder with diluent marker in sample plate, add 24 µL of Blank Solution to unused wells. Place ladder in corresponding 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-476-22
   - DNF-476-33
   - DNF-476-55

   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 Small Fragment DNA Ladder result using the Fragment Analyzer systems with the DNF-476 Small Fragment kit. The peaks are annotated by size (bp). Method: DNF-476-33 (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-476 Small Fragment kit and suggested remedies. Contact Agilent Technical Support if you have any additional troubleshooting or instrument 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 for smear or 60,000 RFU for fragment, or total input DNA concentration does not far exceed recommended limits. | 1 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment. |
| DNA Sample smear overlaps with Lower 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. |
| | 2 Input DNA sample concentration too high. | 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 kit range. | 1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment; OR Prepare fresh sample and analyze with NGS Fragment kit (1 –6,000 bp) (p/n DNF-473-0500 or DNF-473-1000); or HS Large Fragment 50 kb kit (p/n DNF-464-0500). |
| 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; OR Prepare fresh sample and analyze with HS Small Fragment kit (p/n DNF-477-0500). |

| | | |
|---|---|---|
| | 2 Sample not added to DM or not mixed well. | 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. | 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 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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