



Agilent DNF-930 Reagent Kit (75 – 20000 bp) 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 DNF-930 Reagent kit from Agilent is for the analysis of dsDNA fragments between 75 bp and 20 kb. Sizing and relative quantification between samples can be obtained using this kit. Example applications include PCR fragment sizing, and restriction digest analysis.

Specifications

| Analytical specifications | dsDNA 930 assay |
|---|--|
| DNA Sizing Range | 75 bp – 20,000 bp |
| DNA Sizing Accuracy ¹ | + 10% or better |
| DNA Fragment Concentration Range ¹ | 0.5 ng/μL – 50 ng/μL input DNA (adjustable by dilution sample) |
| Separation Resolution | 75 bp – 1,500 bp ≤ 10% (33-55 array) 1,500 bp – 20,000 bp ≤ 15% (33-55 array) |
| DNA Sizing Precision ¹ | 5% CV |

Physical Specifications

| | |
|--------------------------------|--|
| Total electrophoresis run time | 22cm ² : 18 minutes, 33cm: 30 minutes, 55cm: 75 minutes |
| Samples per run | 12, 48 or 96; depending on the instrument type |
| Sample volume required | 2 μL (adjustable depending on sample concentration) |
| Kit stability | 4 months |

¹ Results using DNA Ladder of DNA Fragment standards initially prepared in 1x TE buffer.

² 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 |
|----------------------|-------------------------------|---|------------------|
| 5191-6599* | | dsDNA 930/935, 500, 4°C | |
| | DNF-930-0240 | dsDNA 930 Gel, 240 mL | 1 |
| | DNF-355-0125 | 5x 930 dsDNA Inlet Buffer, 125 mL • Dilute with sub-micron filtered water prior to use | 1 |
| | DNF-495-0060 | Dilution Buffer 1X TE, 60mL | 1 |
| DNF-930-FR* | | dsDNA 930, FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 1 |
| | FS-SLR930-U100 | 1 kb Plus DNA Ladder, 100 µL | 1 |
| | FS-SMK930-0003 | Markers, 75bp & 20kb, 3.2 mL | 1 |
| 5191-6614* | | Qualitative DNA, 500, RT | |
| | FS-SM015 | Mineral Oil Dropper Bottle, 15mL | 1 |
| | DNF-475-0050 | 5x Capillary Conditioning Soln, 50 mL | 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.

Kit Components – 1000 Sample Kit

| Kit Component Number | Part Number (Re-order Number) | Description | Quantity Per Kit |
|----------------------|-------------------------------|--|------------------|
| 5191-6600* | | dsDNA 930/935, 1000, 4°C | |
| | DNF-930-0500 | dsDNA 930 Gel, 500 mL | 1 |
| | DNF-355-0300 | 5x 930 dsDNA Inlet Buffer, 300 mL <ul style="list-style-type: none"> Dilute with sub-micron filtered water prior to use | 1 |
| | DNF-495-0125 | Dilution Buffer 1X TE, 125 mL | 1 |
| <hr/> | | | |
| DNF-930-FR* | | dsDNA 930, FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 2 |
| | FS-SLR930-U100 | 1 kb Plus DNA Ladder, 100 µL | 2 |
| | FS-SMK930-0003 | Markers, 75bp & 20kb, 3.2 mL | 2 |
| <hr/> | | | |
| 5191-6615* | | Qualitative DNA, 500, RT | |
| | FS-SM015 | Mineral Oil Dropper Bottle, 15mL | 1 |
| | DNF-475-0100 | 5x Capillary Conditioning Soln, 100 mL | 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 μ L volumes (sample plates) and 1,000 μ L 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 | <ul style="list-style-type: none"> • Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature |
| Steps before sample preparation | <ul style="list-style-type: none"> • Allow reagents to equilibrate at room temperature for 30 min prior to use |
| Pipetting practice | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • When mixing sample with diluent buffer, it is important to mix the contents of the well thoroughly to achieve the best results. It is highly suggested to perform one of the following methods to ensure complete mixing. Apply a new seal to 96-well plate prior to mixing and centrifugation. 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. • Apply a new seal to 96-well plate prior to mixing and centrifugation • 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 |

75 bp/20,000 bp Marker preparation

- 1 Store the 75 bp and 20 kb Marker solution at -20°C upon arrival.
- 2 Bring the 75 bp and 20 kb Marker solution to room temperature prior to use; vortex solution to ensure it is properly mixed.
- 3 The marker solution is supplied as a ready-to-use solution, containing 0.5 ng/µL of each fragment in 1x TE buffer solution. It is intended for use as an external standard.
- 4 Prepare the marker solution plate by dispensing 30 µL/well into Row A only (12-Capillary) or every well (96-Capillary) of a separate sample plate. Cover the wells with 20 µL/well of the supplied mineral oil to allow reuse for at least 30+ injections. Cover the wells with 20 µL/well of the supplied mineral oil to allow reuse for at least 30+ injections.
- 5 The prepared marker solution plate should be placed into drawer "M" (third from top) of the Fragment Analyzer system. Ensure that the plate is loaded with well A1 toward the back left on the tray.

1 kb Plus DNA Ladder preparation

- 1 Store the 1 kb Plus DNA Ladder at -20°C upon arrival.
- 2 Bring the 1 kb Plus DNA Ladder to room temperature prior to use; vortex solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
1. 3 The 1 kb Plus DNA Ladder is supplied as a concentrate. This enables the solution to be diluted with either 1x TE or 0.1x TE depending upon the available sample concentration and matrix (see section Sample Plate Preparation). The solution contains 50 ng/μL total DNA concentration in a 1x TE buffer solution. It is intended for use as a sizing standard for calibration of DNA size. For optimal sizing results the 1 kb DNA Ladder should be loaded in Well 12 of each row to be analyzed (12-capillary system) or Well H12 (96-capillary system).
- 4 Prepare the working 1 kb Plus DNA Ladder by diluting with either 1x TE buffer or 0.1x TE buffer. Suggested dilutions are:
 - **When working with higher sample concentrations** (total input concentration > 10 ng/ μL): Dilute 1 kb Plus DNA Ladder 12x with Dilution Buffer 1x TE in sample well (2 μL 1 kb Plus DNA Ladder + 22 μL Dilution Buffer 1x TE).
 - **When working with lower sample concentrations** (total input concentration < 10 ng/ μL): Dilute 1 kb Plus DNA Ladder 50x with 0.1x TE buffer in sample well (1 μL 1 kb Plus DNA Ladder + 49 μL 0.1x TE buffer).
- 5 The highest level of sizing accuracy is obtained when the 1 kb Plus DNA Ladder is diluted to a similar concentration range and with a similar diluent (1x or 0.1x TE) to the samples being analyzed.

Sample plate preparation

Some suggested sample preparation guidelines are presented below. It may be necessary to adjust the sample dilution and diluent concentration (1x TE or 0.1x TE) depending upon initial sample concentration and sample matrix. For best results, the 1 kb Plus DNA Ladder should be prepared with a similar concentration and diluent to the samples.

- 1 If total initial sample concentration is > 10 ng/μL (e.g., PCR products):
 - a. Using a clean 96-well sample plate, pipette 22 μL of supplied Dilution Buffer 1x TE solution to each well including any ladder well(s).
 - b. Pipette 2 μL of each DNA sample into the respective wells of the sample plate; mix the contents of the well thoroughly using one of the methods suggested in Mixing and Centrifugation Recommendation.
- 2 If total initial sample concentration is < 10 ng/μL (e.g., restriction digests):
 - a. Prepare a 0.1x TE solution by diluting the supplied Dilution Buffer 1x TE 10x with deionized water.
 - b. Using a clean 96-well sample plate, pipette 20 μL of the 0.1x TE buffer solution to each well to contain sample. Pipette 49 μL of the 0.1x TE buffer solution to any well(s) to contain 1 kb Plus DNA Ladder.
 - c. Pipette 4 μL of each DNA sample into the respective wells of the sample plate; mix the contents of the well thoroughly using one of the methods suggested in Mixing and Centrifugation Recommendation.

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

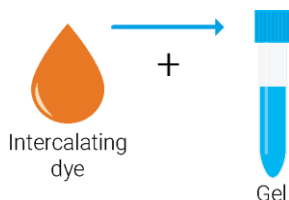
| # of Samples to be Analyzed ¹ | Volume of Intercalating Dye | Volume of Separation Gel ² | Volume of 1x Conditioning Solution ² |
|--|-----------------------------|---------------------------------------|---|
| 96 | 4.0 µL | 40 mL | 40 mL |
| 192 | 8.0 µL | 80 mL | 80 mL |
| 288 | 12.0 µL | 120 mL | 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.

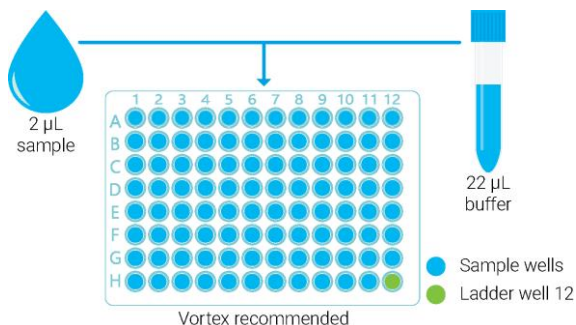
² A 5 mL minimum volume in the tube is included.

Agilent dsDNA 930 (75-20000bp) DNF-930 assay operating procedure

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



- Place a fresh 1x 930 dsDNA Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.
 - 5200 system; Fill row A of buffer plate
 - 5300 system - 48 capillary; Fill rows A-D of buffer plate
 - 5300/5400 system - 96 capillary; Fill all rows of buffer plate
- Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.
 - 5200 system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B"
 - 5300 system - 48 capillary; Fill rows A-D of a sample plate with 100 μ L/well, place in drawer '3'
 - 5300/5400 system - 96 capillary; Fill all rows of a sample plate with 100 μ L/well, place in drawer '3'
 - 5400 system; place in drawer "S"
- Prepare Marker plate and place in drawer 'M' on the system, 30 μ L/well. Add 1 drop or \sim 30 μ L of mineral oil to each well. The marker plate should last for 30+ injections or \sim 1 month.
 - 5200 system; Fill row A of sample plate
 - 5300 system - 48 capillary; Fill rows A-D of sample plate
 - 5300/5400 system - 96 capillary; Fill all rows of sample plate
- Dilute 1 kb Plus DNA Ladder (50 ng/ μ L) to desired working concentration:
 - Dilute 12x (2 μ L ladder per 22 μ L 1x TE buffer) for samples > 10 ng/ μ L
 - Dilute 50x (1 μ L ladder per 49 μ L 0.1x TE buffer) for samples < 10 ng/ μ L
- Mix samples with Diluent Buffer 1x TE Buffer or 0.1x TE Buffer depending on concentration. Place prepared ladder in correct well based on 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

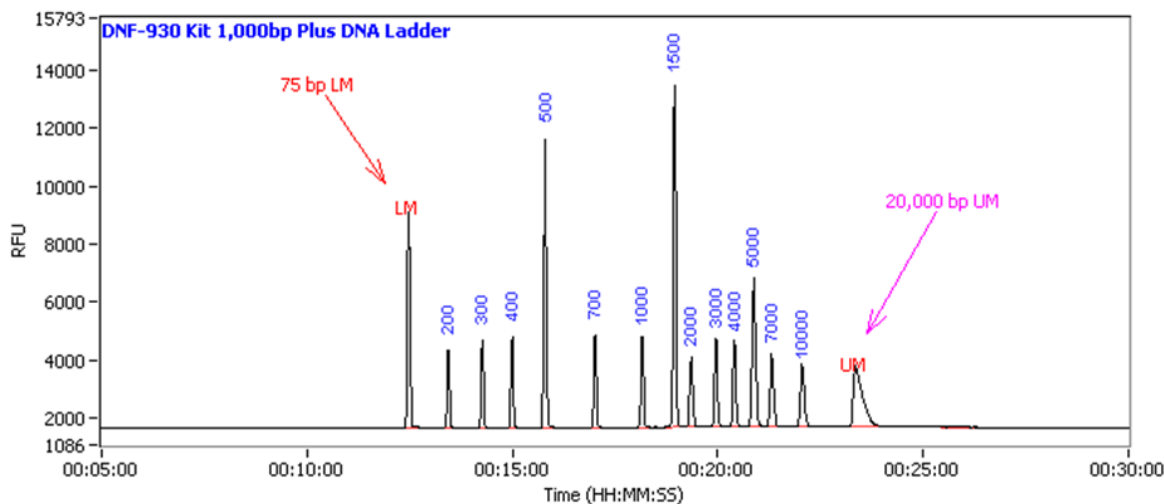
Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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-930-33 – DNA 75-20000 bp **OR** DNF-930-33 – DNA 75-20000 bp-3kv
 - 3.2 DNF-930-55 – DNA 75-20000 bp
4. Enter **Tray Name**, **Folder Prefix**, and **Notes**(optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

1 kb Plus DNA Ladder result



Representative 1 kb Plus DNA Ladder result injected with 75 bp lower marker and 20,000 bp upper marker, using the Fragment Analyzer system with the DNF-930 reagent kit. Array: 33 cm effective, 55 cm total length “short” array. Separation: 8 kV, 30 min.

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-930 Reagent 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 is too high. | 1 Further dilute input DNA sample concentration with 1x TE buffer and repeat experiment. 2 Reduce injection time and/or injection voltage and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy. |
| Sample peak(s) migrate before or co-migrate with 75 bp Lower Marker. | 1 Excess primer-dimer species in sample. | 1 Further dilute input DNA sample concentration with 1x TE buffer to minimize primer-dimer interference and repeat experiment. 2 If fragment size is below 1,500 bp or 5,000bp, analyze using DNF-910 Reagent kit(DNF-910-K0500; 35bp – 1,500 bp range) or DNF-915 Reagent kit (DNF-915-K0500; 35 bp – 5,000 bp) to better resolve primer-dimer species. |
| Sample peak(s) migrate after or co-migrate with 20,000 bp Upper Marker. | 1 DNA sample size out of range of assay. | 1 Analyze samples with Genomic DNA Analysis kit (no upper marker), or the Large Fragment DNA Analysis kits, which have a large upper size limit.). |
| No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed. | 1 Sample concentration too low and out of range. 2 Sample was not added to 1x TE diluent or not mixed well. | 1 Prepare more concentrated sample and repeat experiment (e.g. 4 µL sample + 20 µL DI water); OR Repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate. 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 sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 µL is required. | 1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 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. If no liquid is observed follow the steps outlined in the Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.

For Research Use Only

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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