Agilent dsDNA 930 Reagent Kit (75-20000 bp)

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 dsDNA 930 Reagent kit from Agilent is for the analysis of dsDNA fragments between 75 bp and 20,000 bp. Sizing and relative quantification of samples can be obtained using this kit. Example applications include general PCR fragment sizing and QC, microsatellite/SSR analysis, and genotyping.

Specifications

Analytical Specifications	dsDNA 930 Reagent Kit
DNA Sizing Range	75 bp – 20,000 bp
DNA Sizing Accuracy[1]	± 10% or better
DNA Sizing Precision[1]	5% CV
Separation Resolution	75 bp – 1,500 bp ≤ 10%; 1,500 bp – 20,000 bp ≤ 15% (short capillary array, 33 cm)
Fragment Concentration Range[1]	0.5 ng/µL – 50 ng/µL input DNA (adjustable by dilution of sample)
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)
Guaranteed Shelf Life	4 months

1 Results using DNA Ladder or DNA Fragment standards initially prepared in 1X TE buffer.
2 The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

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
5191-6599*		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	1
	DNF-495-0060	Dilution Buffer 1X TE, 60 mL	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, 75 bp & 20 kb, 3.2 mL	1
5191-6614*		Qualitative DNA, 500, RT	
	FS-SMO15	Mineral Oil Dropper Bottle, 15 mL	1
	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
5191-6600*		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	1
	DNF-495-0125	Dilution Buffer 1X TE, 125 mL	1
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, 75 bp & 20 kb, 3.2 mL	2
5191-6615*		Qualitative DNA, 1000/5000, RT	
	FS-SMO15	Mineral Oil Dropper Bottle, 15 mL	1
	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 available from Fisher Scientific 14-432-22 or VWR 21008-940)
    - 250 mL for 5300 and 5400 Fragment Analyzer systems (Corning 430776 available from 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	• 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, dilution buffer, or Marker remains within or on the outside of the tip.

Marker Plate Preparation	• Bring ready-to-use Marker solution to room temperature prior to use. • Agitate and centrifuge ready-to-use Marker solution to mix. • Dispense 30 µL/well of Marker into wells of a separate sample plate. (12 capillary; row A, 48 capillary; rows A-D, 96 capillary; all rows) • Cover the wells with 1 drop or 30 µL of mineral oil to allow reuse for at least 30+ injections. • Place plate in drawer M. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate.
1 kb Plus DNA Ladder Preparation	• The 1 kb Plus DNA Ladder solution is supplied as a concentrate. Prepare the working 1 kb Plus DNA Ladder solution by diluting with either 1x TE buffer of 0.1x TE buffer: When working with higher sample concentrations (>10 ng/µL): Dilute 1 kb Plus DNA Ladder solution 12x with 1x TE buffer in sample well (2 µL ladder + 22 µL 1x TE buffer) When working with lower sample concentrations (<10 ng/µL): Dilute 1 kb Plus DNA Ladder solution 50x with 0.1x TE buffer in sample well (1 µL ladder + 49 µL 0.1x TE buffer) • 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 to the samples being analyzed.
Sample Plate Preparation	If total initial sample concentration is > 10 ng/µL (A) : • Pipette 22 µL of 1x TE dilution buffer to each well to contain sample or ladder. • Pipette 2 µL of each DNA sample into the respective wells of the sample plate. Mix contents of the well using a pipette by aspiration or expulsion in the pipette tip. • Pipette 2 µL of 1 kb Plus DNA Ladder solution into the respective well(s) of the sample plate to contain ladder. Mix contents of the well using the pipette by aspiration/expulsion in the pipette tip. If total initial sample concentration is < 10 ng/µL (B) : • Prepare a 0.1x TE solution by diluting the supplied 1x TE buffer 10x with deionized water. • 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 to contain 1 kb Plus DNA Ladder • Pipette 4 µL of each DNA sample into the respective wells of the sample plate. Mix contents of the well using the pipette by aspiration/expulsion in the pipette tip. • Pipette 1 µL of 1 kb Plus DNA Ladder solution into the respective well(s) of the sample plate to contain ladder. Mix contents of the well using the pipette by aspiration/expulsion in the pipette tip. • Place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm for 2 minutes. Ensure there is no well-to-well transfer of samples when vortexing. • 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. • Input range of concentration should not exceed 20,000 RFU.

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

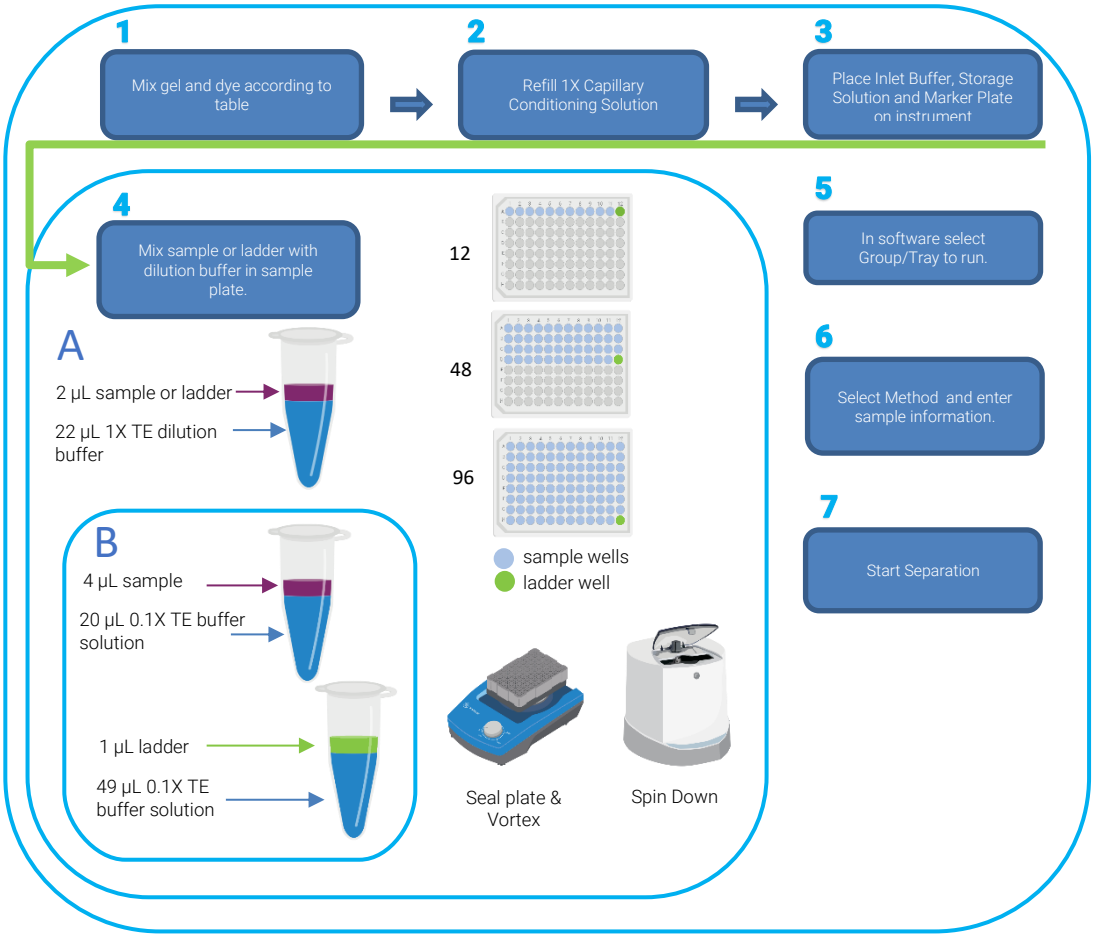
Agilent dsDNA 930 Reagent Kit Operating Procedure

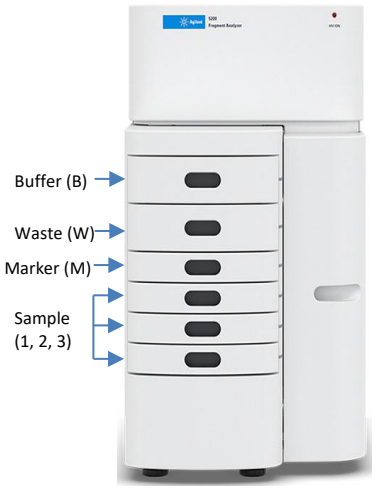
1. Mix fresh gel and dye according to the volumes in the preparation tables.
2. Refill 1X Capillary Conditioning Solution as needed.
3. Inspect and empty, if necessary, waste plate located in drawer "W".
4. Place a fresh 1X Inlet Buffer tray, 1 mL/well, in drawer "B". Replace daily.
   - 5200 – row A
   - 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

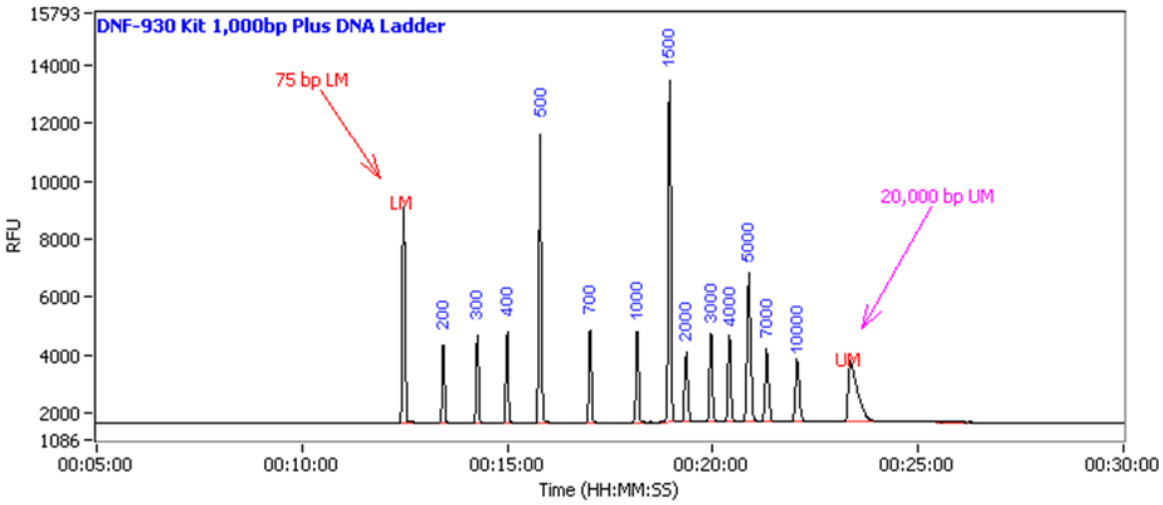
   Prepare Marker plate and place in drawer "M", 30 µL/ well. Add 1 drop or ~30 µL of mineral oil to each well. The Marker plate should last for 30+ injections or ~1 month.
   - 5200 – Row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows
5. Mix samples with (**A**) 1x OR (**B**) 0.1x TE dilution buffer in sample plate, depending on concentration. Refer to sample plate preparation. Add 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-930-22
   - DNF-930-33
   - DNF-930-55

   Enter Tray Name, Folder Prefix and Notes, if desired.
8. Add method to the queue by selecting "OK", press play to start the separation.





1 kb Plus DNA Ladder result



Representative 1 kb Plus DNA Ladder result co-injected with 75 bp lower marker and 20,000 bp upper marker, using the Fragment Analyzer system with the dsDNA 930 Reagent kit. Method: DNF-930-33 ("short" array). RFU values may differ between instruments.

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the dsDNA 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 >> 60,000 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.
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 5,000 bp, analyze using DNF-915 dsDNA 915 Reagent kit (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 Depending upon sample size, analyze samples with DNF-464 HS Large Fragment 50kb Kit (75 bp – 48,5000 bp), or a Genomic DNA Analysis kit which contains no upper marker (e.g., DNF-467 Genomic DNA 50 kb kit or DNF-468 HS Genomic DNA 50 kb kit).
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 dilution buffer or not mixed well.	1 Prepare more concentrated sample and repeat experiment (e.g., 4 µL sample + 20 µL TE buffer). 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.	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 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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Not for use in diagnostic procedures.

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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