

Agilent Plasmid DNA 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 Plasmid DNA Kit (DNF-940) from Agilent is for the analysis of supercoiled plasmid DNA between 2,000 bp and 10,000 bp. Sizing of the supercoiled plasmid and relative quantification of samples can be obtained using this kit. The DNF-940 kit is capable of accurately sizing the supercoiled plasmid DNA form only. Linearized plasmid DNA can also be detected, but will migrate more slowly and not size accurately, as it is referenced to the supercoiled Plasmid DNA Ladder included with this kit. The nicked/open circular form of plasmid DNA cannot be detected or analyzed with the DNF-940 kit. Example applications include supercoiled plasmid quality control and/or sizing.

Specifications

Analytical Specifications	Plasmid DNA Kit
DNA Sizing Range	2,000 bp – 10,000 bp
DNA Sizing Accuracy ¹	± 10%
DNA Fragment Concentration Range ¹	0.1 ng/μL – 1.0 ng/μL Final Concentration DNA in 1X TE Buffer
Physical Specifications	
Total Electrophoresis Run Time	33cm: 30 minutes
Samples Per Run	12, 48 or 96; depending on the instrument type
Sample Volume Required	Variable (24 μL final sample volume required; optimal sample concentration range in 1X TE Buffer)
Guaranteed Shelf Life	4 months

¹ Results obtained using plasmid DNA Ladder or supercoiled DNA sample prepared in 1X TE buffer.

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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
5191-6601*		Plasmid DNA, 500, 4 °C	
	DNF-940-0240	Plasmid DNA 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-940-FR*		Plasmid DNA FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	FS-SLR940-0001	Plasmid DNA Ladder, 1.2 mL	1
	FS-SMK940-0003	Plasmid DNA Marker, 3.2 mL	2
5191-6614*		Qualitative DNA, 500, RT	
	FS-SM015	Mineral Oil Dropper Bottle, 15 mL	1
	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 Short	A2300-1250-3355
5300 Fragment Analyzer.	FA 48 Capillary Array Short FA/ZAG 96 Capillary Array Short	A2300-4850-3355 A2300-9650-3355
5400 Fragment Analyzer	FA/ZAG 96 Capillary Array Short	A2300-9650-3355

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

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	<ul style="list-style-type: none"> Ambient operating temperature: 19 – 25 °C (66 – 77 °F) Keep instrument reagents at room temperature during sample preparation.
Sample Input Concentration	<ul style="list-style-type: none"> Ensure sample input concentrations lie within kit specifications. Sample signal should not exceed 60,000 RFU.
Steps before sample preparation	<ul style="list-style-type: none"> Allow instrument reagents to equilibrate at room temperature for 30 min prior to use. Spin tubes after thawing to ensure liquid is at the bottom of the tube.
Pipetting practice	<ul style="list-style-type: none"> Pipette reagents against the side of the 96-well sample plate or sample tube. Ensure no sample, dilution buffer, or PDM solution remains within or on the outside of the tip.
Marker Plate Preparation	<ul style="list-style-type: none"> Bring ready-to-use Plasmid DNA Marker (PDM) solution to room temperature before use. Agitate and centrifuge ready-to-use PDM solution to mix. Dispense 30 µL/well of PDM solution 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. Place plate in drawer M. If not using right away, cover and keep in the dark at 4°C, warm to RT and centrifuge before running plate. The PDM solution is light and temperature sensitive. Once prepared and covered with mineral oil, the solution may be used for up to one week. When not stored on the instrument, the plate should be stored in the dark.
Sample Plate Preparation	<ul style="list-style-type: none"> Pipette 24 µL of each sample diluted with 1X TE dilution buffer to the concentration range specified. If running the DNA Ladder with samples, pipette 24 µL Ladder solution (ready-to-use) directly into the specified ladder well of the sample plate or row to be analyzed. Fill any unused wells within the sample row or plate with 24 µL of 1X TE dilution buffer. 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.

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

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Agilent Plasmid DNA 940 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 tray, 1 mL/well, in drawer "B". Replace daily.


- 5200 – row A
- 5300 – 48 capillary, rows A-D
- 5300/5400 – 96 capillary, all rows

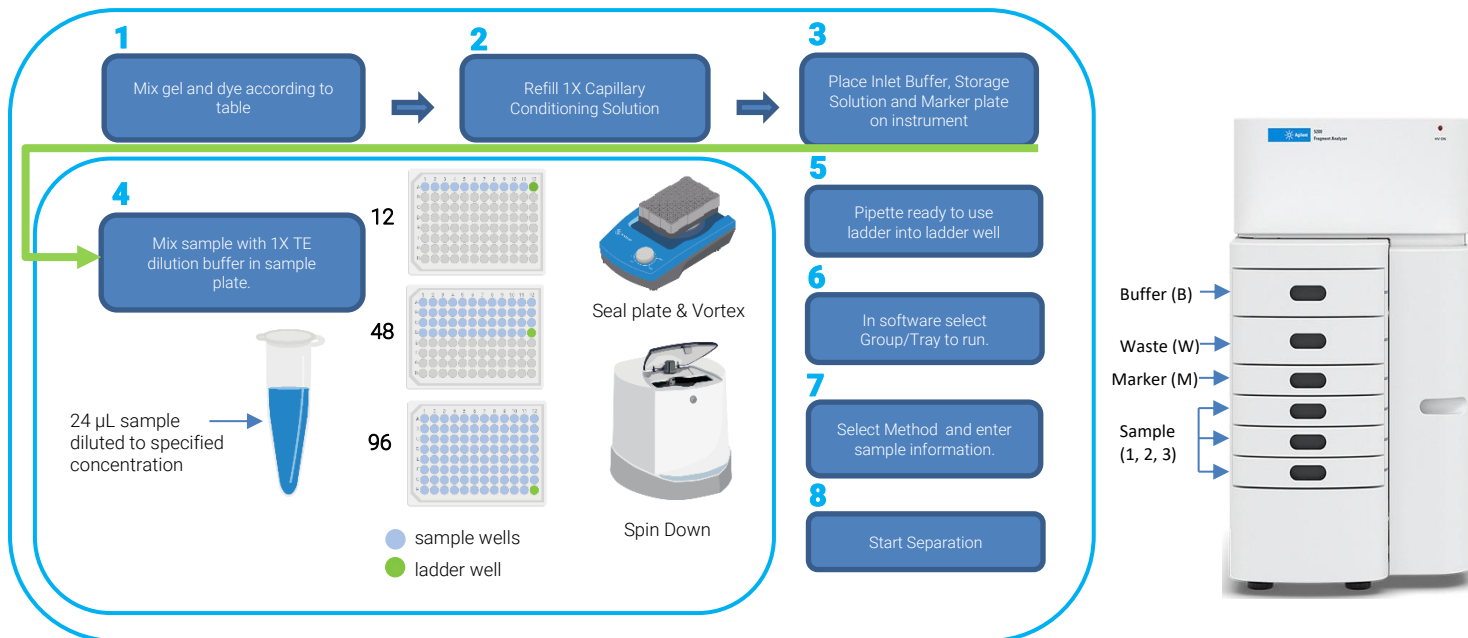
Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.

- 5200 – row H, 1 mL/well, drawer B
- 5300 – 48 capillary, rows A-D, 100 μ L/well, drawer 3
- 5300/5400 – 96 capillary, all rows, 100 μ L/well, drawer 3

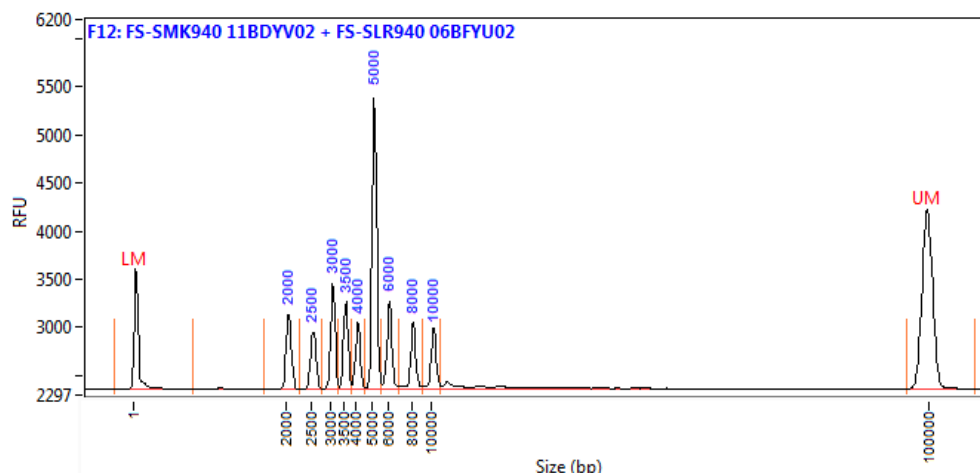
Prepare Marker plate and place in drawer "M", 30 μ L/ well. Add 1 drop or \sim 30 μ L of mineral oil to each well. The marker plate should last for 30+ injections or \sim 1 month. 5200 – Row A

- 5300 – 48 capillary, rows A-D
- 5300/5400 – 96 capillary, all rows

5. Add samples to sample plate.
6. Add ready to use ladder in corresponding well (see sample plate image below), depending on capillary array used.
7. Select Row/Group/Tray to run. Enter sample ID and Tray ID, if desired.
8. Add to queue, from the dropdown select the corresponding method based on your capillary length;
 - DNF-940-33
 Enter Tray Name, Folder Prefix and Notes, if desired.
9. Add method to the queue by selecting "OK", press play  to start the separation.



Plasmid DNA Ladder result



Representative Plasmid DNA Ladder result using the Fragment Analyzer system with the Plasmid DNA Analysis kit. Peaks annotated by size (bp). RFU values may differ between instruments.

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-940 Plasmid DNA Analysis 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. Ensure peak height does not exceed 10,000 RFU (fragment) or total input concentration does not exceed recommended limits.	1 Further 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 was not added to 1x TE dilution buffer or not mixed well.	1 Prepare more concentrated sample and repeat experiment (e.g., 4 μ L sample + 20 μ L 1X 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. 2 Insufficient sample volume. A minimum of 20 μ L 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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