Agilent

Agilent ZAG-135 dsDNA Kit

Quick Guide for the ZAG DNA Analyzer System

For Research Use Only. Not for use in diagnostic procedures

This Quick Guide is intended for use with the Agilent ZAG DNA Analyzer system only. The ZAG-135 dsDNA kit is designed for analyzing double-stranded DNA fragments from 35 to 1,500 base pair.

Specifications

Analytical specifications[1,2]	dsDNA 135 assay
DNA Sizing Range	35 bp – 1,500 bp
DNA Sizing Accuracy[2]	± 5% or better
DNA Sizing Precision[2]	2% CV
DNA Fragment Concentration Range[1]	0.5 ng/µL – 50 ng/µL input DNA (adjustable by dilution sample)
Separation Resolution	100 – 1,500 bp ≤ 10% (33-55 array) 100 – 1,500 bp ≤ 10% (55-80 array)
Physical Specifications	
Total Electrophoresis Run Time	33cm: 20 minutes, 55cm: 35 minutes
Samples Per Run	96-Capillary: 95 (+1 DNA Ladder Well) or 96 (Imported DNA Ladder)
Sample Volume Required	2 µL
Guaranteed Shelf Life	4 months

1 Results using DNA ladder in 1X TE buffer.
2 Results using DNA samples in 1X TE buffer.

Kit Components- 5000 Sample Kits

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6610*		ZAG 130/135 dsDNA, 5000, 4°C	
	ZAG-130-0500	ZAG 130 dsDNA Separation Gel, 500mL	1
	DNF-495-0125	Dilution Buffer 1x TE, 125mL	1
	DNF-355-0500	5x 930 dsDNA Inlet Buffer, 500 mL • Dilute with sub-micron filtered water prior to use	1
ZAG-135-FR*		ZAG 135 dsDNA, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	FS-SLR910-0001	100 bp DNA Ladder, 1mL • 100 bp – 1,000 bp; 2.5ng/µL total DNA concentration in 1X TE Buffer	2
	FA-MRK910F-0003	Markers, 1bp & 1,500bp 10% Formamide, 3.2mL • Lower Marker (set to 1 bp) and Upper Marker (1,500 bp; at 0.5 ng/µL) in 1x TE buffer with 10% Formamide	1
5191-6615*		Qualitative DNA, 1000/5000, RT	
	DNF-475-0100	5x Capillary Conditioning Soln, 100mL	1
	FS-SMO15	Mineral Oil Dropper Bottle, 15mL	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.

WARNING

Working with Chemicals

- **Refer to product safety data sheets for further information**
- **When working with the ZAG DNA Analyzer kit components follow the appropriate safety procedures such as wearing personal protective equipment (PPE).**

Additional Material Required for Analysis with the ZAG DNA Analyzer System

Instrument	Compatible Arrays	Part Number
ZAG DNA Analyzer	ZAG 96-Capillary Array Short, 33 cm	A2300-9650-3355
ZAG DNA Analyzer	ZAG 96-Capillary Array Long, 55 cm	A2300-9650-5580

Software

- Agilent ZAG DNA Analyzer controller software
- ProSize data analysis software

Reagents

- Capillary Storage Solution (GP-440-0100)

Additional equipment/reagents required (not supplied)

- 96-well PCR sample plates. Please refer to Appendix – ZAG DNA Analyzer Compatible Plates and Tubes in the ZAG DNA 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
- Adhesive PCR plate seals
- 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
- 250 mL conical: 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)

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 19 – 25°C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
Sample Input Concentration	• Ensure sample input concentrations lie within kit specifications. • Sample signal should not exceed 60,000 RFU.
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 TE, 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 to the 22 µL of TE, 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 the plate with foil seal and keep at 4°C, warm to RT and centrifuge before running the 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.

# of 96-well plate Analyzed[1]	Intercalating Dye Volume (µL)	Separation Gel Volume (mL)
1 (1 FC Only)	2.5 µL	25 mL
2 (1 FC +1 GP)	3.0 µL	30 mL
5 (1 FC + 4 GP)	4.5 µL	45 mL
8 (1FC + 7GP)	6.0 µL	60 mL
10 (1 FC + 9 GP)	7.5 µL	75 mL

FC=Full Conditioning
GP = Gel Prime Only

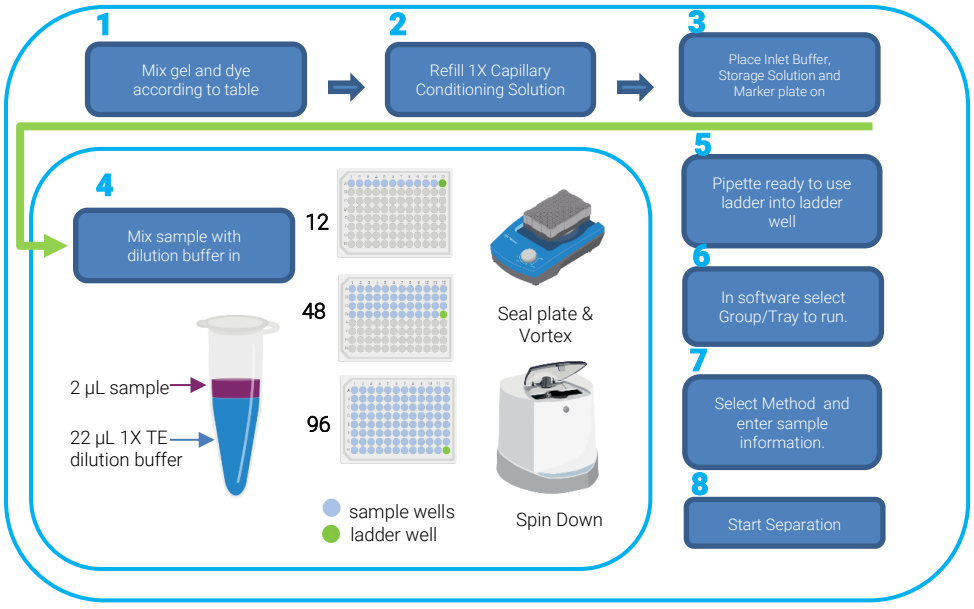
Conditioning Solution

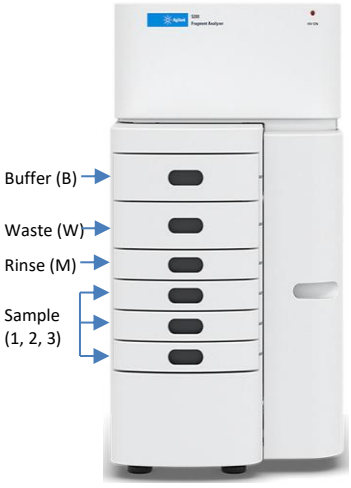
The provided 5X Conditioning Solution must be diluted to 1X using submicron DI water prior to use. Invert to mix.

# of 96-well plates to be Analyzed[1]	Volume of 1X Conditioning Solution (mL)
1 (1 FC Only)	40 mL
2 (1 FC +1 GP)	80 mL
5 (1 FC + 4 GP)	120 mL
8 (1FC + 7GP)	160 mL
10 (1 FC + 9 GP)	200 mL

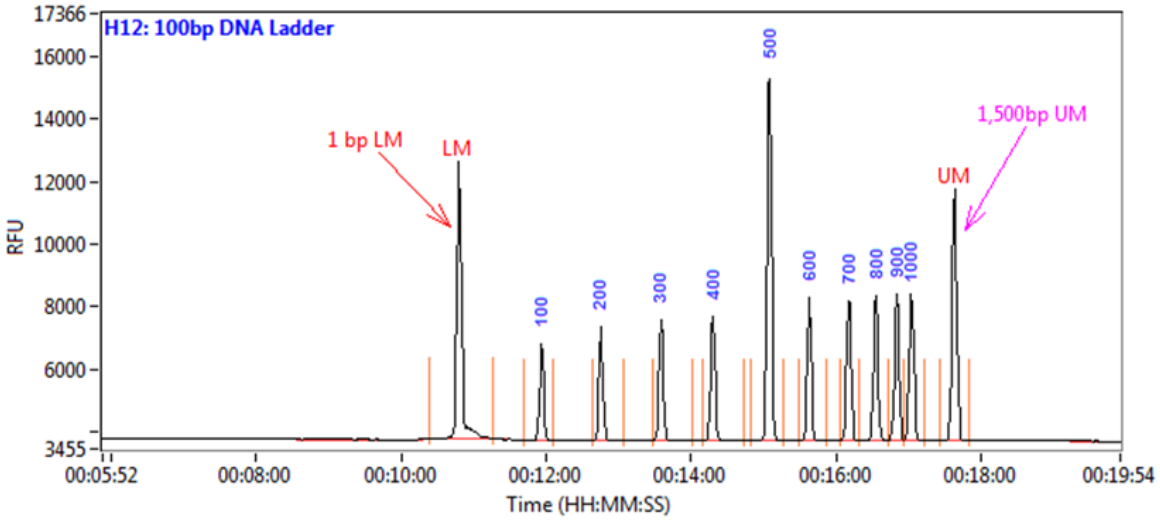
Agilent ZAG-135 dsDNA assay operating procedure

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.
   a. ZAG system - 96 capillary; Fill all rows of buffer plate
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.
   a. ZAG system - 96 capillary; Fill all rows of a sample plate with 100 µL/well, place in drawer 'S'
4. Place Marker plate in drawer 'M' on the system, 30 µL/well with 30 µL overlay (one drop) of Mineral Oil. The marker plate should last for 30+ injections or ~1 month.
   a. ZAG system - 96 capillary; Fill all rows of sample plate
5. Mix samples with Diluent Buffer 1X TE in sample plate, add 24 µL of 100 bp DNA Ladder ("ready to use"; no dilution) into well H12.
6. Select Row, Group or Tray to run.
7. Enter **sample ID** and **Tray ID**(optional).
8. Select **Add to Queue**, from the dropdown menus select the corresponding method based on your capillary length;
   a. ZAG135FC33 – DNA 1-1500bp – Full Conditioning
   b. ZAG135GP33 – DNA 1-1500bp – Gel Prime Only
   c. ZAG135FC55 – DNA 1-1500bp – Full Conditioning
   d. ZAG135FP55 – DNA 1-1500bp – Gel Prime Only
9. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
10. Select **OK** to add method to the queue.
11. Select ▶ to start the separation.





100 bp DNA Ladder result



Representative 100 bp DNA Ladder result injected with 1 bp lower marker and 1,500 bp upper marker, using the ZAG system with the ZAG 135 dsDNA Kit. Method: ZAG135FC33 (short array).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the ZAG 135 dsDNA kit (1-1,500 bp) 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 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment; OR Repeat experiment using decreased injection time (e.g., 10 sec); OR prepare fresh sample using ZAG 135 dsDNA (1-500 bp) (Part # ZAG-135)
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 + 20 µLDI Water) OR Repeat experiment with increased injection time. 2 Verify sample was correctly added and mixed in sample well.
Sample peak(s) migrate before or co-migrate with 1 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.
Sample peak(s) migrate after of co-migrate with 1,500 bp Upper Marker.	1 DNA sample size out of range of assay.	1 Analyze samples with ZAG 110 dsDNA kit, or ZAG-130 dsDNA kit.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of the sample plate 2 Insufficient sample volume. A minimum of 20 µL 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 Appendix – Capillary Array Cleaning of ZAG User 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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