Agilent ZAG-110 dsDNA Kit Quick Guide for the ZAG DNA Analyzer System

The Agilent ZAG DNA Analyzer system is an automated capillary electrophoresis platform for scalable, flexible, fast, and reliable electrophoresis of DNA fragments.

This Quick Guide is intended for use with the Agilent ZAG DNA Analyzer system only. The ZAG-110 dsDNA assay is designed for analyzing double-stranded DNA fragments from 35 to 5,000 base pair.

Specifications

Analytical specifications[1,2]	dsDNA 110 assay
DNA Sizing Range	35 bp – 5,000 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	35 – 100 bp ≤ 10%, 100 – 1,000 bp ≤ 5%, 1,000 – 5,000 bp ≤ 10% (33-55 array) 35 – 1,500 bp ≤ 5%, 1,500 – 5,000 bp < 10% (55-80 array)
Physical Specifications	
Total electrophoresis run time	33cm: 60 minutes, 55cm: 80 minutes
Samples per run	96-Capillary: 95 (+1 DNA Ladder Well) or 96 (Imported DNA Ladder)
Sample volume required	2 µL
Kit stability	4 months

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

Kit Components – 5000 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6609*		ZAG 115 dsDNA (35-5,000bp), 5000, 4°C	
	ZAG-110-0500	ZAG 110 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-110-FR*		ZAG 110 dsDNA, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	FS-SLR915-0001	100bp DNA Plus Ladder, 1mL • 100 bp – 3,000 bp; 2.5ng/µL total DNA concentration in 1X TE Buffer	2
	FA-SMK480-0003	35bp and 5,000bp Markers, 3.2mL • 0.5ng/µL concentration each in 1x TE buffer	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

WARNING

- 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 goggles, safety gloves and protective clothing.

Additional Material Required for Analysis with the ZAG DNA Analyzer Systems

- ZAG DNA Analyzer systems with LED fluorescence detection:
- ZAG DNA Analyzer system (p/n M5320AA)
  - ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
  - ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580):
- Agilent ZAG DNA Analyzer controller software (Version 1.0 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 – 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
- 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)
- Capillary Storage Solution (p/n GP-440-0100)

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
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 1x 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 1x 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

Prepare gel/dye mixture for ZAG DNA Analyzer System. 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.

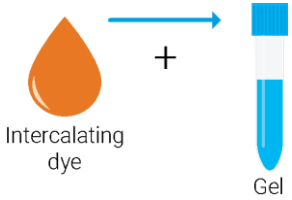
NOTE: Centrifuge dye prior to opening the vial to reduce risk of leaking.

ZAG DNA Analyzer system volume specifications for 96-capillary

# of 96-well plates to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel[2]	Volume of 1x Conditioning Solution[2]
1 (1 FC Only)	2.5 µL	25 mL	40 mL
2 (1 FC +1 GP)	3.0 µL	30 mL	80 mL
5 (1 FC + 4 GP)	4.5 µL	45 mL	120 mL
8 (1FC + 7GP)	6.0 µL	60 mL	160 mL
10 (1 FC + 9 GP)	7.5 µL	75 mL	200 mL
FC=Full Conditioning GP = Gel Prime Only			

Agilent ZAG 110 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.

    2.1. ZAG system - 96 capillary; Fill all rows of buffer plate

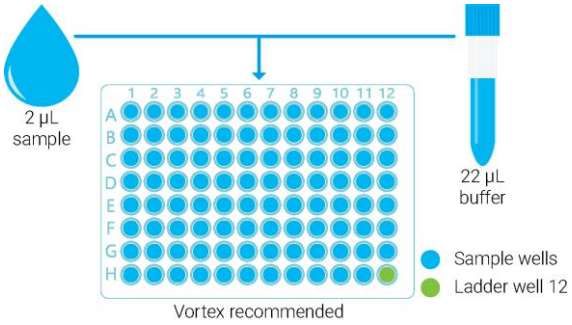
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

    3.1. 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.

    4.1. 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 Plus Ladder ("ready to use"; no dilution) into well H12.



ZAG system - 96 capillary; Ladder – well H12

WARNING

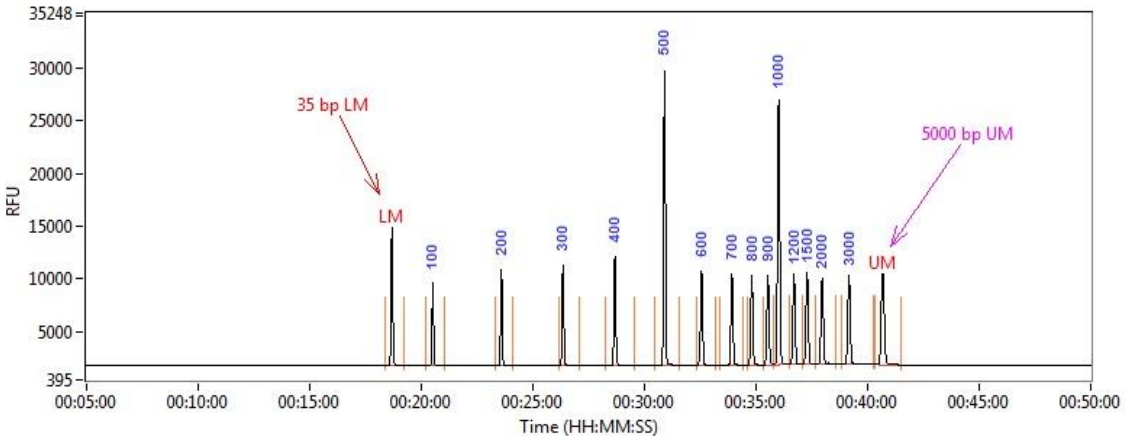
Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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 ZAG DNA 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 ZAG110FC33 – DNA 35-5,000bp – Full Conditioning
   - 3.2 ZAG110GP33 – DNA 35-5.000bp – Gel Prime Only
   - 3.3 ZAG110FC55 – DNA 35-5,000bp – Full Conditioning
   - 3.4 ZAG110FP55 – DNA 35-5,000bp – Gel Prime Only
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

100 bp Plus DNA Ladder result



Representative 100 bp Plus DNA Ladder result injected with 35 bp lower marker and 5,000 bp upper marker, using the *ZAG* system with the *ZAG*-110-5000 reagent kit. Method: **ZAG110FC33** (short array).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the ZAG 110 dsDNA kit (35-5,000 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 >> 20,000 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 110 dsDNA (35-5,000 bp) (Part # ZAG-110)
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. (e.g. 4 µL + 20 µLDI Water) OR Repeat experiment with increased injection time and/or injection voltage for Marker and Sample Plates.
	2 Sample was not added to 1x TE diluent or not mixed well	2 Verify sample was correctly added and mixed in sample well.
Sample peak(s) migrate before or co-migrate with 35 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 5,000 bp Upper Marker.	1 DNA sample size out of range of assay.	1 Analyze samples with ZAG 130 dsDNA kit (75bp – 20,000bp)
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of the sample plate	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. If no liquid is observed, follow the steps outlined in the Appendix – Capillary Array Cleaning of ZAG 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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