Agilent ZAG-130 dsDNA Kit Quick Guide for ZAG DNA Analyzer Systems

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-130 dsDNAkit is designed for analyzing double-stranded DNA fragments from 75 to 20,000 basepair.

Specifications

Analytical specifications[1,2]	dsDNA 130 assay
DNA Sizing Range	75 bp – 20,000 bp
DNA Sizing Accuracy[2]	± 10% or better
DNA Sizing Precision[2]	5% CV
DNA Fragment Concentration Range[1]	0.5 ng/µL – 50 ng/µL input DNA (adjustable by dilution sample)
Separation Resolution	75 – 1,500 bp ≤ 10%, 1,500 – 20,000 bp ≤ 15%
Physical Specifications	
Total electrophoresis run time	33cm: 30 minutes, 55cm: 75 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
5191-6610*		ZAG 130/135 dsDNA Kit, 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-130-FR*		ZAG 130 dsDNA, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	2
	FS-SLR930-U100	1,000 Plus DNA Ladder, 100µL • 75 bp – 20,000 bp; 50 ng/µL total DNA concentration in 1X TE Buffer	2
	FA-SMK930-0003	75 bp and 20,000 bp Markers, 3.2 mL • 0.5 ng/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 system 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 or ladder 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 and keep at 4°C, warm to RT and centrifuge before running plate

Gel preparation

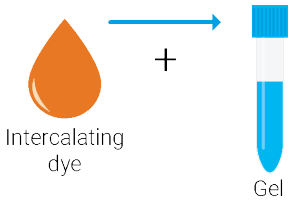
Prepare gel/dye mixture for ZAG DNA Analyzer System. To ensure the gel/dye mixture is mixed homogeneously without generating bubbles, gently invert the centrifuge tube 5 to 10 times, depending on the volume of the mixture.

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 130 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.

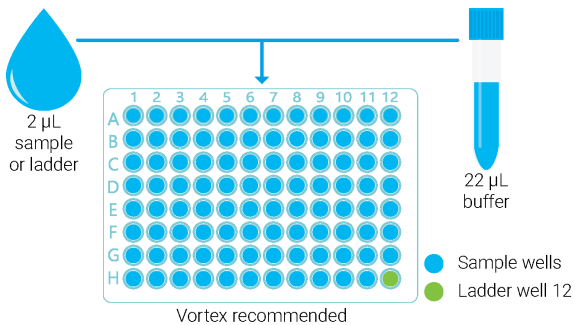
   4.1. ZAG system - 96 capillary; Fill all rows of sample plate

5. Dilute 1 kb Plus DNA Ladder (50 ng/µL) to desired working concentration:

   5.1 Dilute 12x (2 µL ladder per 22 µL 1x TE buffer) for samples > 10 ng/µL.

   5.2 Dilute 50x (1 µL ladder per 49 µL 0.1x TE buffer) for samples < 10 ng/µL.

6. Mix samples with Diluent Buffer 1X or 0.1x TE depending on the concentration. Place prepared ladder in ladder well.



ZAG system - 96 capillary; Ladder – well H12

WARNING

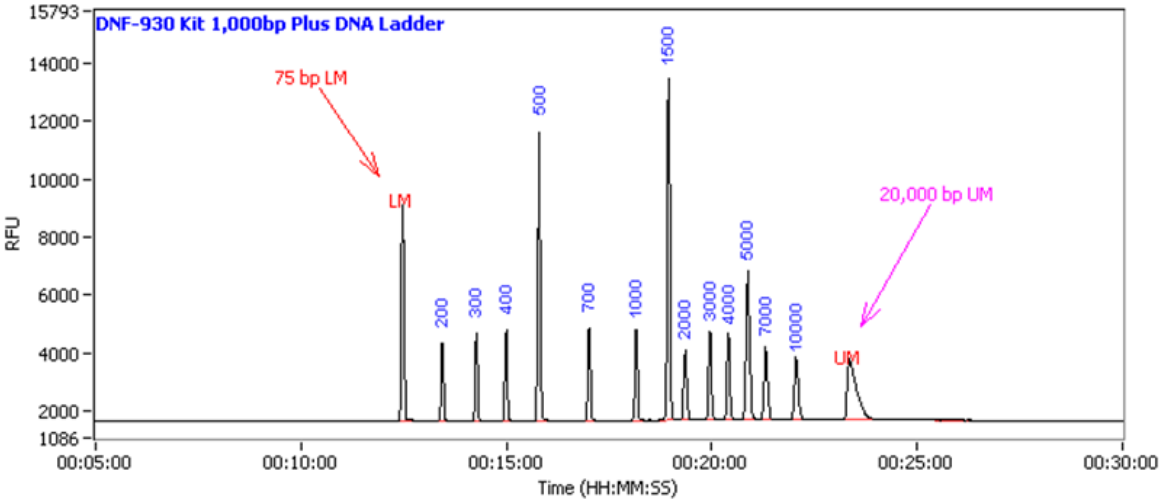
Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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 ZAG130FC33 – DNA 75-20000bp – Full Conditioning
   - 3.2 ZAG130GP33 – DNA 75-20000bp – Gel Prime Only
   - 3.3 ZAG130FC55 – DNA 75-20000bp – Full Conditioning
   - 3.4 ZAG130FP55 – DNA 75-20000bp – 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.

1,000 bp DNA Ladder result



Representative 1,000 bp Plus DNA Ladder result injected with 75 bp lower marker and 20,000 bp upper marker, using the ZAG system with the ZAG-130-5000 reagent kit. Method: **ZAG130FC33** (short array).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the ZAG 130 dsDNA kit (75-20000 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 130 dsDNA (1-500 bp) (Part # ZAG-130)
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 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 ZAG-110 dsDNA Kit (35 bp – 5,000 bp) to better resolve primer-dimer species.
Sample peak(s) migrate after of co-migrate with 20,000 bp Upper Marker.	1 DNA sample size out of range of assay.	1 Fragment samples if possible and reanalyze.
Poor resolution of ladder peaks. Slower migration time than expected.	1 Capillary Array Vent Valve is partially plugged with gel.	1 Inspect and if necessary clean Capillary Array Vent Valve as described in the ZAG Troubleshooting and Maintenance Guide.

Technical Support and Further Information

For technical support, please visit www.agilent.com. It offers useful information, support and current developments about the products and technology.

www.agilent.com

Edition 08/20

SD-AT000147