Agilent ZAG 130 dsDNA Kit (75 - 20000 bp)

Kit Guide

For Research Use Only.
Not for use in diagnostic procedures.
Notices

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Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
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Agilent ZAG 130 dsDNA Kit

The ZAG-130 Reagent Kit from Agilent (5,000 Samples)(Part # ZAG-130-5000) is for the analysis of dsDNA fragments between 75 bp and 20,000 bp. Sizing and relative quantification between samples can be obtained using this kit. Example applications include PCR fragment sizing, and restriction digest analysis.

<table>
<thead>
<tr>
<th>Table 1 Physical Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>Sample Volume Required</td>
</tr>
<tr>
<td>Number of Samples per Run</td>
</tr>
<tr>
<td>Total Electrophoresis Run Time</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Analytical Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>DNA Sizing Range</td>
</tr>
<tr>
<td>Separation Resolution</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>DNA Sizing Precision(^1)</td>
</tr>
<tr>
<td>DNA Sizing Accuracy (^1)</td>
</tr>
<tr>
<td>DNA Fragment Concentration Range (^1)</td>
</tr>
</tbody>
</table>

\(^1\) Using DNA Ladder or DNA Fragment standards prepared in 1x TE buffer.
Table 3  Storage Conditions

<table>
<thead>
<tr>
<th>Store at –20°C:</th>
<th>Store at 4°C (DO NOT FREEZE):</th>
<th>Store at Room Temperature (DO NOT FREEZE):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercalating Dye</td>
<td>dsDNA Gel</td>
<td>5x Capillary Conditioning Solution</td>
</tr>
<tr>
<td>Markers, 75 bp &amp; 20 kb, 3.2mL</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>Mineral Oil</td>
</tr>
<tr>
<td>1,000bp Plus DNA Ladder</td>
<td>Dilution Buffer 1x TE</td>
<td></td>
</tr>
</tbody>
</table>

Ensure all reagents are completely warmed to room temperature prior to use.

Table 4  Kit Components

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZAG-130-0500</td>
<td>ZAG 130 dsDNA Separation Gel</td>
<td>500 mL</td>
</tr>
<tr>
<td>DNF-600-U030</td>
<td>Intercalating Dye</td>
<td>30 μL x 2</td>
</tr>
<tr>
<td>DNF-355-0500</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>500 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-475-0100</td>
<td>5x Capillary Conditioning Solution</td>
<td>100 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>FS-SMK930-0003</td>
<td>75 bp and 20,000 bp Markers</td>
<td>3.2 mL</td>
</tr>
<tr>
<td></td>
<td>• 0.5 ng/μL concentration each in 1x TE buffer</td>
<td></td>
</tr>
<tr>
<td>FS-SLR930-U100</td>
<td>1,000 bp Plus DNA Ladder</td>
<td>100 μL x 2</td>
</tr>
<tr>
<td></td>
<td>• 75 bp – 20,000 bp; 50 ng/μL total DNA concentration in 1x TE buffer</td>
<td></td>
</tr>
<tr>
<td>DNF-495-0125</td>
<td>Dilution Buffer 1x TE</td>
<td>125 mL</td>
</tr>
<tr>
<td>FS-SMO15</td>
<td>Mineral Oil E</td>
<td>15 mL</td>
</tr>
</tbody>
</table>

Conical 250 mL Centrifuge Tube for prepared Separation Gel/Intercalating Dye mixture

**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.
Additional Material and Equipment Required

Material and Equipment Required for Analysis with the ZAG DNA Analyzer

Hardware:
  • ZAG DNA Analyzer 96-capillary CE system with LED fluorescence detection – (Part # M5320AA)
    o FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355), OR
    o FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)

Software:
  • ZAG instrument control software (Version 1.0 or higher)
  • ProSize data analysis software (Version 2.0.0.61 or higher)

Reagents:
  • Capillary Storage Solution, 100 mL (Part #GP-440-0100)
Additional Material and Equipment Required

Additional Equipment/Reagents Required (Not Supplied)

- 96-well PCR sample plates. Please refer to Appendix C – ZAG Compatible Plates and Tubes in the ZAG 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 Solutions)
- Fisherbrand 96 DeepWell 1mL Plate, Natural Polypropylene, part # 12-566-120 (Inlet Buffer and waste plate)
- Reagent Reservoir, 50 mL (VWR 82026-355 or similar) (for use in pipetting Inlet Buffer plates/sample trays)
- Conical centrifuge tubes for prepared dsDNA Gel/Dye mixture and/or 1x Capillary Conditioning Solution
  - 250 mL (for 96-Capillary instruments or larger volumes): Corning #430776, available from Fisher #05-538-53 or VWR #21008-771
  - 50 mL (for 12-Capillary instruments or 50 mL volumes): BD Falcon #352070, available from Fisher #14-432-22 or VWR #21008-940
- Clean graduated cylinder (for measurement of dsDNA Gel volume and dilution of 5x 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solution)
3 Agilent ZAG 130 dsDNA Kit Protocol

Gel Preparation

1. Store the dsDNA Separation Gel at 4°C upon arrival.
2. The Intercalating Dye is supplied as a 20,000X concentrate in DMSO and should be stored at -20°C.

**NOTE**
For this assay, it is recommended to use the Intercalating Dye at 2X normal concentration (1:10,000 dilution).

3. Bring the dsDNA Separation Gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and dsDNA Separation Gel necessary for less than two weeks of operation. Use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment. For maximum accuracy, it is recommended to dispense dsDNA Separation Gel into a clean glass graduated cylinder for volume measurement and transfer to the working tube prior to adding Intercalating Dye.

**NOTE**
Some loss of detection sensitivity will be observed over a two-week period after the gel/dye mixture has been prepared. For maximum detection sensitivity applications, it is recommended to prepare gel/dye mixture daily. It is not recommended to use gel/dye mixture that is more than two weeks old.

Two different capillary flushing methods are employed with the kit (Figures 6-7):
- **Full Conditioning**, and
- **Gel Prime Only**

It is recommended to run the **Full Conditioning** method every ten (10) experiment cycles, when switching from a different Separation Gel kit, or when the instrument has not been run for several days. The Full Conditioning flush consumes approximately 15 mL of Separation Gel and 20 mL of Capillary Conditioning Solution per cycle.

The **Gel Prime Only** method may be used in between Full Conditioning cycles. The Gel Prime Only flush consumes approximately 5 mL of Separation Gel per cycle.
Approximate Separation Gel volumes required for varying sample amounts are listed below.

### Table 5  Volume Specifications for 96-capillary systems

<table>
<thead>
<tr>
<th># of 96-well plates to be analyzed</th>
<th>Approximate Volume of ZAG dsDNA Gel</th>
<th>Volume of Intercalating dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1 FC Only)</td>
<td>25 mL</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>2 (1 FC + 1 GP)</td>
<td>30 mL</td>
<td>3.0 µL</td>
</tr>
<tr>
<td>5 (1 FC + 4 GP)</td>
<td>45 mL</td>
<td>4.5 µL</td>
</tr>
<tr>
<td>8 (1 FC + 7 GP)</td>
<td>60 mL</td>
<td>6.0 µL</td>
</tr>
<tr>
<td>10 (1 FC + 9 GP)</td>
<td>75 mL</td>
<td>7.5 µL</td>
</tr>
</tbody>
</table>

FC = Full Conditioning, GP = Gel Prime Only.

5. Place the prepared dsDNA separation gel/intercalating dye mixture onto the instrument and insert into the desired gel fluid line (Gel 1 or Gel 2 pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.

6. When adding separation gel to the instrument, update the solution levels in the ZAG instrument control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1). The user will be prompted to scan the associated barcode from the dsDNA Separation Gel, scan the barcode and select OK.
Agilent ZAG 130 dsDNA Kit Protocol

7 When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh gel/dye mixture. From the main screen of the ZAG Instrument control software, select **Utilities > Prime...** Select the desired fluid line(s) (Conditioning, Gel 1, or Gel 2) and press **OK** to purge the fluid line with fresh gel (Figure 2).

![Prime menu](image)

**Figure 2.** Prime menu

Inlet Buffer Preparation

1 Store the 5x 930 dsDNA Inlet Buffer at 4°C upon arrival. Do not freeze.

2 Bring the 5x 930 dsDNA Inlet Buffer to room temperature prior to mixing and use.

3 In a clean container, add 20 mL of the 5x 930 dsDNA Inlet Buffer per 80 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at 4°C if desired.
Capillary Conditioning Solution Preparation

1. Store the 5x Capillary Conditioning Solution at room temperature upon arrival. Do not freeze or refrigerate.

2. In a clean container (e.g. 250 mL conical centrifuge tube), add 20 mL of the 5x Capillary Conditioning Solution per 80 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at room temperature if desired.

3. Once mixed, place the 1x Capillary Conditioning Solution onto the instrument and insert the conditioning fluid line (Conditioning Solution pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.

4. The 1x Capillary Conditioning Solution should be added to the system as use demands. A typical Full Conditioning experiment cycle consumes approximately 20 mL.

5. When adding fresh 1x Capillary Conditioning Solution to the instrument, update the solution levels in the ZAG Instrument control software and scan the corresponding barcode. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).
Instrument Preparation

1. Check the fluid level of the waste bottle and waste tray daily and empty as needed.

2. Prepare a fresh 96 DeepWell 1mL Plate filled with 1.0 mL/well of 1x 930 dsDNA Inlet Buffer daily. Do not overfill the wells of the inlet buffer plate.

3. Place the prepared inlet buffer plate into Drawer “B” (top drawer) of the ZAG. Ensure that the plate is loaded with well A1 toward the back left on the tray.

4. Place an empty 96 DeepWell 1mL Plate into Drawer “W” (second from top) of the ZAG. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.

5. In Drawer “S”, place a sample plate filled with 100 μL/well of Capillary Storage Solution (Part # GP-440-0100). Drawer S is used for the Store location, and the array moves to this position at the end of the experimental sequence.

   **NOTE**

   Ensure the Drawer “S” location is always filled with Capillary Storage Solution, and the capillary array is placed against Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

Marker/Ladder/Sample Preparation

General Information

1. The recommended 96-well sample plate for use with the ZAG system is a semi-skirted PCR plate from Eppendorf (Part #951020303). Please refer to Appendix C – ZAG Compatible Plates and Tubes in the ZAG User Manual for a complete approved sample plate list. The system has been designed to operate using these dimensions/styles of PCR plates.

   **NOTE**

   The use of PCR plates with different dimensions to the above recommended plate could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.
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75 bp/20,000 bp Marker Preparation
1. Store the 75 bp and 20,000 bp Marker solution at -20°C upon arrival.
2. Bring the 75 bp and 20,000 bp Marker solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
3. The Marker solution is supplied as a ready-to-use solution, containing 0.5 ng/μL of each fragment in a 1x TE buffer solution. It is intended for use as an external standard marker plate.
4. Prepare the Marker solution plate by dispensing 30 μL/well into a separate sample plate. Cover the wells with 20 μL/well of the supplied mineral oil to allow reuse for at least 50+ injections.
5. The prepared Marker solution plate should be placed into Drawer “M” (third from top) of the ZAG. Ensure that the plate is loaded with well A1 toward the back left on the tray.

1,000 bp Plus DNA Ladder Preparation
1. Store the 1,000 bp Plus DNA Ladder solution at -20°C upon arrival.
2. Bring the 1,000 bp Plus DNA Ladder solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
3. The 1,000 bp Plus DNA Ladder solution is supplied as a concentrate. This enables the solution to be diluted with either 1x TE or 0.1x TE depending upon the available sample concentration and matrix (see section Sample Plate Preparation below). The solution contains 50 ng/μL total DNA concentration in a 1x TE buffer solution. It is intended for use as a sizing standard for calibration of DNA size. For optimal sizing results the 1,000 bp Plus DNA Ladder should be loaded in Well H12 of the sample plate.
4. Prepare the working 1,000 bp Plus DNA Ladder solution by diluting with either 1x TE buffer or 0.1x TE buffer. Suggested dilutions are:
   - **When working with higher sample concentrations** (total initial sample concentration > 10 ng/μL): Dilute 1,000 bp Plus DNA Ladder solution 12X with 1x TE buffer in sample well (2 μL 1,000 bp Plus DNA Ladder + 22 μL 1x TE buffer).
   - **When working with lower sample concentrations** (total initial sample concentration < 10 ng/μL): Dilute 1,000 bp Plus DNA Ladder solution 50X with 0.1x TE buffer in sample well (1 μL 1,000 bp Plus DNA Ladder + 49 μL 0.1x TE buffer).
5 The highest level of sizing accuracy is obtained when the 1,000 bp Plus DNA Ladder is diluted to a similar concentration range (yielding similar peak height RFU values) and with a similar diluent (1x or 0.1x TE) to the samples being analyzed.

Sample Plate Preparation

1 Some suggested sample preparation guidelines are presented below. It may be necessary to adjust the sample dilution and diluent concentration (1x TE or 0.1x TE) depending upon initial sample concentration and sample matrix. For best results, the 1,000 bp Plus DNA Ladder should be prepared with a similar concentration and diluent to the samples.

2 If total initial sample concentration is > 10 ng/μL (e.g., PCR products):
   - Using a clean 96-well sample plate, pipette 22 μL of supplied 1x TE buffer solution to each well that is to contain sample or ladder.
   - Pipette 2 μL of each DNA sample into the respective wells of the sample plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
   - 1,000 bp Plus DNA Ladder: Pipette 2 μL of 1,000 bp Plus DNA Ladder solution into well H12.

3 If total initial sample concentration is < 10 ng/μL (e.g., restriction digests):
   - Prepare a 0.1x TE solution by diluting the supplied 1x TE buffer 10X with deionized water.
   - Using a clean 96-well sample plate, 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 well H12, to contain 1,000 bp Plus DNA Ladder.
   - Pipette 4 μL of each DNA sample into the respective wells of the sample plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
   - 1,000 bp Plus DNA Ladder: Pipette 1 μL of 1,000 bp Plus DNA Ladder solution into well H12; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
**Agilent ZAG 130 dsDNA Kit Protocol**

**NOTE**

**Important Sample Mixing Information**

When mixing sample with diluent solution, 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:

- **When adding 2 µL of sample to the 22 µL of diluent,** swirl the pipette tip while pipetting up/down to further mix.
- **After adding 2 µL of sample to the 22 µL of diluent,** place a plate seal on the sample plate and **vortex the sample plate at 3000 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 diluent,** **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 volume. Some models enable using the pipette tip for both adding and mixing.

4. After mixing sample/1,000 bp Plus DNA Ladder and diluent solution in each well, centrifuge the plate to remove any air bubbles. Check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. The presence of trapped air bubbles can lead to injection failures.

5. Run the sample plate immediately once prepared, or cover the sample plate with a cover film, store at 4°C, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (20 µL/well).

6. To run the samples, place the plate in one of the two available sample plate trays (Drawers 4-5 from the top) of the ZAG instrument. Load or create the experimental method as described in the following sections.

7. The CCD detection system of the ZAG system provides a high dynamic range for detection. An ideal injection range would yield peak heights from 100 – 20,000 RFUs. Overloading of sample can decrease separation resolution and saturate the detector, leading to mismatched lower/upper marker peak heights and poor results. It is important to optimize sample dilution and concentration and use experimental parameters to work within the specified RFU range. The highest level of sizing accuracy is obtained when the sample and DNA Ladder peak heights are of similar RFU peak heights.

**TIP**

If the above methods yield peak heights consistently above 20,000 RFUs, decrease the marker/sample injection time or reduce the sample volume to 1 µL sample + 23 µL 1x TE.
Agilent ZAG 130 dsDNA Kit Protocol

8 If low signals are encountered, increase the marker/sample injection time, or alternatively add 4 μL of sample + 20 μL of DI water in each well. When making adjustments to the sample dilution, the total volume should be maintained to at least 24 μL.

NOTE
Whenever making adjustments to the sample dilution, ensure the Dilution Factor of the ProSize software is adjusted accordingly when processing the data.
Performing Experiments

Running an Experiment

1. To set up an experiment, from the main screen of the ZAG Instrument control software, select the Operation tab (Figure 3). Select the sample tray location to be analyzed (1, 2, ...9) by left clicking the Sample Tray # dropdown or by clicking the appropriate sample plate tab (alternate plate view) and choosing the appropriate location.

2. Left click a well of the desired sample plate row with the mouse. The selected row will be highlighted in the plate map (e.g., Row A in Figure 3). Enter the sample name if desired into the respective Sample ID cell by left clicking the cell and typing in the name. Alternatively, sample information can be imported from .txt or .csv file by selecting the Load from File... option.

Figure 3. Main screen showing selection of sample row and entering sample information
Agilent ZAG 130 dsDNA Kit Protocol

3 After sample information for the row or plate has been entered, under the Run Tray field press Add to queue. The Separation Setup form will be displayed enabling the user to select the experimental method and enter additional information (Figure 4).

![Separation Setup form](image)

Figure 4. Separation Setup form to select experimental Method and enter tray/folder information

4 In the Separation Setup pop-up form, left click the dropdown and select the appropriate preloaded experimental Method file. The available methods are sorted by kit number and are linked to the directory containing methods for the currently installed capillary array length (e.g., 33cm or 55cm). Select the following method:

- Select ZAG130FC33 – DNA 75-20000bp – Full Conditioning.mthds when the 33 cm effective, 55 cm total “short” capillary array is installed and the user needs to perform the Full Conditioning method.
- Select ZAG130GP33 – DNA 75-20000bp – Gel Prime Only.mthds when the 33 cm effective, 55 cm total “short” capillary array is installed to perform the Gel Prime Only method.
- Select ZAG130FC55 – DNA 75-20000bp – Full Conditioning.mthds when the 55 cm effective, 80 cm total “long” capillary array is installed and the user needs to perform the Full Conditioning method.
- Select ZAG130GP55 – DNA 75-20000bp – Gel Prime Only.mthds when the 55 cm effective, 80 cm total “long” capillary array is installed to perform the Gel Prime Only method.

NOTE A Full Conditioning Method should be run every 10 injections, when switching from a different Separation Gel kit, or when the instrument has not been run for several days. A Gel Prime Only Method is run in between Full Conditioning Methods.

5 Select the appropriate Gel line being used for the experiment (Gel 1 or Gel 2) using the dropdown.
The Tray Name can be entered to identify the sample plate. The Folder Prefix if entered will amend the folder name (normally a time stamp of HH-MM-SS from the start of the CE run).

To copy the experimental results to another directory location in addition to the default save directory, check the Copy results box and select the desired Copy path: directory by clicking the “…” button and navigating to the desired save directory.

Any Notes can be entered regarding the experiment; they will be saved and displayed in the final PDF report generated by the ProSize software.

Once all information has been entered, press OK to add the method to the instrument queue (press Cancel to abort adding the method).

Repeat Steps 1-9 for any remaining sample rows to be analyzed.

After a sample tray has been added to the queue, the method(s) will be listed on the main screen under the Method Queue field (Figure 5).

Prior to starting the experiment, verify all trays (buffer/storage, waste, marker, sample, etc.) have been loaded into their respective drawer locations.

Press the Play icon ( ) to start the sequence loaded into the queue. To Pause the queue after the currently running experiment is completed, press the button. To Clear the run queue of all loaded experiments, press the button.

Figure 5. Main Screen after selection of samples to the run queue
Agilent ZAG 130 dsDNA Kit Protocol

14 Once an experiment has been loaded onto the queue, the user can view or edit the method (Administrator level only can edit a method) by pressing the Method Summary field. To remove the method from the queue, press the X button; to view the stepwise details of the method press the double down arrow icon.

15 The user may add a Pause or Prime step into the queue by right clicking the mouse while over the queue and selecting Insert Pause or Insert Prime.

16 The order of the experimental queue can be rearranged by dragging down individual entries. Further information regarding the Method Queue operation is provided in the ZAG User Manual.

17 Once started, the instrument will perform all the programmed experiments in the Method Queue uninterrupted unless a pause step is present. Note that additional experiments can be programmed and added to the Method Queue at any time while the instrument is running if desired. After completion of the last queued experiment, the stage will automatically move to the Store location. (96-Capillary Systems: typically set to the Sample 3 location).
Agilent ZAG 130 dsDNA Kit Protocol

Viewing and Editing Experimental Methods

1. A User level operator can View the steps of the experimental method by pressing the View link on the Separation Setup screen, or by pressing the Method Summary option once a method has been loaded onto the experimental queue. User level operators cannot edit any steps of a queued separation method.

2. Administrator level operators can Edit certain steps of the experimental method. To open the method editor screen, press the Edit link from the Separation Setup screen (Figure 4). The method editor screen is displayed, showing the steps of the method (Figure 6).

3. The preloaded, optimized steps for the ZAG130FC33 (Figure 6) and the ZAG130GP33 (Figure 7) methods are shown below. The general steps of the method are as follows:
   a. Full Condition or Gel Prime flushing method (Automatically enabled). Gel Selection: Gel 1.
   b. Perform Prerun (enabled) (8 kV, 30 sec)
   c. Rinse (disabled)
   d. Marker Injection (enabled) Voltage Injection (5 kV, 5 sec). This step injects the 75 bp/20,000 bp marker plate.
   e. Rinse (disabled)
   f. Sample Injection (enabled) Voltage Injection (5 kV, 5 sec). This step injects the prepared sample plate.
   g. Separation (enabled) Voltage (8 kV, 30 min). This step performs the CE Separation.
Figure 6 ZAG130FC33 – DNA 75-20000bp – Full Conditioning method
Figure 7. ZAG130GP33 – DNA 75-20000bp – Gel Prime Only method
Agilent ZAG 130 dsDNA Kit Protocol

4 Figures 8-9 show the preloaded methods ZAG130FC55 (Figure 8) and ZAG130GP55 (Figure 9), for the 55 cm effective, 80 cm total length "long" array. The prerun and separation voltages are set to 8 kV, the injection voltages to 7.5 kV, and the separation time to 75 min.

Figure 8. ZAG130FC55 – DNA 75-20,000 bp – Full Conditioning method method
An Administrator level user has the option to adjust the Gel Selection; Prerun settings; Rinse settings including Tray and # Dips; Marker Injection settings; Sample Injection settings, and the Separation settings. For example, if the Administrator wants to increase the Separation Voltage and reduce the Separation Time for an experiment, this can be adjusted prior to or while the method is loaded on the experimental queue.

To apply any adjustments to the method being placed on the experimental queue, press the OK button. To exit the editor screen without applying any changes press the Cancel button.

**NOTE** Any edits made to the experimental method from the Separation Setup or Method Summary screen will only apply to the currently loaded experiment in the queue. No changes are made to the original separation method file.
Agilent ZAG 130 dsDNA Kit Protocol

Processing Experimental Data

1. When processing data, the ProSize software will automatically recognize the separation method performed and apply the appropriate configuration file from the C:\ProSize 3.0\Configurations directory:
   - The ZAG130FC33 separation method will be processed using the ZAG130FC33 - DNA 75-20000bp - Full Conditioning.ini configuration file.
   - The ZAG130GP33 separation method will be processed using the ZAG130GP33 - DNA 75-20000bp - Gel Prime Only.ini configuration file.
   - The ZAG130FC55 separation method will be processed using the ZAG130FC55 - DNA 75-20000bp - Full Conditioning.ini configuration file.
   - The ZAG130GP55 separation method will be processed using the ZAG130GP55 - DNA 75-20000bp - Gel Prime Only.ini configuration file.

   NOTE
   If the preloaded ProSize software configuration files listed above are not located in the C:\ProSize 3.0\Configurations or C:\ProSize 2.0\Configurations directory, contact Agilent Technical Support to obtain the files.

2. The data is normalized to the lower marker (set to 75 bp) and upper marker (set to 20,000 bp) and calibrated to the 1,000 bp Plus DNA Ladder run in parallel to the samples. Figure 10 shows an example of the 75 bp and 20,000 bp markers injected with the 1,000 bp Plus DNA Ladder. A total of 15 peaks should be observed.

3. When processing data, the ProSize configuration is set to the DNA mode in the Advanced Settings. The Quantification settings should be set to Use Lower Marker for quantification with a Final Conc. (ng/μL) of 0.5 and a Dilution Factor of 12 (2 μL sample + 22 μL Diluent Marker).

   NOTE
   If a pre-dilution was performed prior to the experiment, the Dilution Factor setting should be changed to accurately reflect the final sample concentration.

4. For full information on processing data, refer to the ProSize User Manual.
ZAG DNA Analyzer Shut Down/Storage

Instrument Shut Down/Storage

The instrument automatically places the capillary array in the Store position against Capillary Storage Solution (Sample Tray 3) after each experiment; no further action is required.

If the instrument is to be idle for more than one day, it is recommended to turn off the power to the system.
4 Checking Your Separation Results

1,000 bp Plus DNA Ladder

Figure 10 shows the typical expected results for the 1,000 bp Plus DNA Ladder diluted 12X with 1x TE diluent, co-injected with the 75 bp lower marker and 20,000 bp upper marker using a 33 cm effective, 55 cm total length capillary array. A total of 15 peaks should be observed, with the sizes annotated as in Figure 10. All fragments in the ladder should be resolved.

Figure 11 shows the same separation performed on a 55 cm effective, 80 cm total length array.

![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).](image)
Figure 11. 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: ZAG130FC55 (long array).
5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the ZAG-130-5000 Reagent Kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

<table>
<thead>
<tr>
<th>Issue</th>
<th>Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>The peak signal is &gt;&gt; 20,000 RFU; upper marker peak is low or not detected relative to lower marker.</td>
<td>1 Input DNA sample concentration is too high.</td>
<td>1 Further dilute input DNA sample concentration with 1x TE buffer and repeat experiment.</td>
</tr>
<tr>
<td></td>
<td>2 Reduce injection time and/or injection voltage, and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy.</td>
<td></td>
</tr>
<tr>
<td>No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.</td>
<td>1 Sample concentration too low and out of range.</td>
<td>1 Prepare more concentrated sample and repeat experiment (e.g. 4 uL sample + 20 uL DI water); or repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate.</td>
</tr>
<tr>
<td></td>
<td>2 Sample was not added to 1x TE diluent or not mixed well.</td>
<td>2 Verify sample was correctly added and mixed to sample well.</td>
</tr>
<tr>
<td>Sample peak(s) migrate before or co-migrate with 75 bp Lower Marker.</td>
<td>1 Excess primer-dimer species in sample.</td>
<td>1 Further dilute input DNA sample concentration with 1x TE buffer to minimize primer-dimer interference, and repeat experiment.</td>
</tr>
<tr>
<td></td>
<td>2 If fragment size is below 5,000 bp, analyze using Agilent ZAG 110 dsDNA Kit (35 bp – 5,000 bp) to better resolve primer-dimer species.</td>
<td></td>
</tr>
<tr>
<td>Sample peak(s) migrate after or co-migrate with 20,000 bp Upper Marker.</td>
<td>1 DNA sample size out of range of assay.</td>
<td>1 Fragment samples if possible and reanalyze.</td>
</tr>
<tr>
<td>Poor resolution of ladder peaks. Slower migration time than expected.</td>
<td>1 Capillary Array Vent Valve is partially plugged with gel.</td>
<td>1 Inspect and if necessary clean Capillary Array Vent Valve as described in the ZAG Troubleshooting and Maintenance Guide.</td>
</tr>
</tbody>
</table>
In This Book

This Kit Guide describes the following:

- Agilent ZAG 130 dsDNA Kit
- Additional Material and Equipment Required
- Agilent ZAG 130 dsDNA Kit Protocol
- Checking Your Separation Results
- Troubleshooting