Agilent Genomic DNA 165 kb Kit Quick Guide for the Femto Pulse System

The Agilent Femto Pulse system is an automated capillary electrophoresis platform for scalable, flexible, fast, and reliable electrophoresis of nucleic acids.

This Quick Guide is intended for use with the Agilent Femto Pulse system only. The Genomic DNA 165 kb kit (275 Samples) (Part # FP-1002-0275) is designed for the pulsed-field CE separation, sizing and quantitation of high molecular weight, low concentration DNA smears and/or fragments from 1.3 kb though 165 kb.

Specifications

Analytical specifications	Genomic DNA 165Kb assay
Sizing Range	1.3 kb – 165 kb
Sizing Accuracy[1]	± 15%
Sizing Precision[1]	20% CV
DNA Fragment Concentration Range[1]	0.3 pg/µL - 30 pg/µL input DNA
DNA Smear Concentration Range[2]	5 pg/µL - 500 pg/µL input DNA
gDNA Quantification Precision	25% CV
Maximum gDNA Concentration	500 pg/µL
Physical Specifications	
Total electrophoresis run time	70 minutes (Fast Method), 180 minutes (Extended Method)
Samples per run	12-Capillary: 11 (+1 Ladder Well)
Sample volume required	2 µL
Kit stability	4 months

[1] Result based on Lambda DNA Fragment.

[2] Result determined using Corielle gDNA sample (Cat# 19238).

Kit Components – 275 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6604*		Femto Pulse, 4°C	
	FP-5001-0250	FP Large DNA Separation Gel, 250 mL	1
	DNF-306-0005	BF-P25 Blank Solution, 5 mL	1
	DNF-325-0075	5x Inlet Buffer, 75 mL	1
	DNF-498-0012	Dilution Buffer 0.25x TE, 12mL	1
	DNF-497-0060	0.25x TE Rinse Buffer, 60mL	1
FP-1002-FR*		Genomic DNA 165 kb, FR	
	FP-6001-U030	FP Intercalating Dye, 30 µL	1
	FP-8001-0003	FP gDNA Diluent Marker, 3 mL	3
	FP-7002-U035	FP 165 kb Ladder, 35 µL	1
5191-6617*		Genomic DNA 165 kb, 275, RT	
	C27-130	Eppendorf LoBind 0.5 mLTubes (Bag of 50)	1
	C280-101	Wide-Bore Genomic Pipette Tips, 1 Box	1
	DNF-425-0050	5x Conditioning Solution, 50 mL	1
	GP-435-0100	Storage Solution, 100 mL	1

*Not Orderable.

WARNING

- Refer to product safety data sheets for further information
- When working with the Femto Pulse assay follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Additional Material Required for Analysis with the Femto Pulse System

- Femto Pulse system with LED fluorescence detection:
- Femto Pulse system (p/n M5330AA)
    - FP 12-Capillary Array, 22 cm (p/n A1600-1250-2240)
- Agilent Femto Pulse controller software (Version 1.0 or higher)
- Agilent ProSize Data Analysis software (Version 3.0 or higher)

Additional equipment/reagents required (not supplied)

- 96-well PCR sample plates. Please refer to Appendix – Femto Pulse Compatible Plates and Tubes in the Femto Pulse 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
- Wide-Bore Genomic pipette tips, Thermo Scientific #21-402-157, as needed for pipetting gDNA samples
- 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
    - 50 mL (Femto Pulse system): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
    - 250 mL (Femto Pulse system or larger volumes): Corning #430776, available from Fisher Scientific #05-538-53 or VWR #21008-771
- 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

Marker/Ladder/Sample Preparation

General Information

1. The recommended 96-well sample plate for use with the Femto Pulse system is a semi-skirted PCR plate from Eppendorf (#951020303). Please refer to the Appendix – Femto Pulse Compatible Plates and Tubes in the Femto Pulse User Manual for a complete approved sample plate list. The system has been designed to operate using these dimensions/styles of PCR plates.
2. Store the gDNA Diluent Marker solution and the 165 kb Ladder at -20°C upon arrival.
3. Allow the marker and ladder solution to warm up to room temperature prior to use. Briefly spin the tubes after thawing to ensure liquid is at the bottom of the tube.

   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.

165kb Ladder Handling and Storage

Prior to first use, the 165 kb Ladder should be aliquoted to minimize the number of freeze/thaw cycles.

1. Equilibrate the 165 kb Ladder to room temperature for about 30 min and mix by gently pipetting up-and-down up to 3 times with wide-bore genomic pipette tips (provided with kit) and a pipettor set to a 15 µL volume. Do not vortex of flick the Ladder tube. Only wide-bore genomic pipette tips should be used to mix the 165 kb Ladder solution.
2. Aliquot the 165 kb Ladder solution into 7 aliquots, 5 µL each, using the provided wide-bore genomic tips and the provided Eppendorf LoBind 0.5 mL tubes. Each aliquot can be used up to 4 times (1 µL per use).
3. Store the 165 kb Ladder aliquots at -20°C. Store the in-use vial of the 165 kb Ladder at 2-8°C for up to 2 weeks. Avoid freeze-thawing of the 165 kb Ladder more than 4 times (additional freeze-thawing may result in degradation of the higher molecular weight fragments in the 165 kb Ladder).

165 kb Ladder Working Solution

1. Before use, equilibrate the 165 kb Ladder aliquot to room temperature for about 30 min.
2. In an Eppendorf LoBind 0.5 mL tube (provided), aliquot 9 µL of the DNF-498 0.25x TE Dilution Buffer.
3. To the same Eppendorf tube aliquot 90 µL of the FP-8001 gDNA Diluent Marker solution. Mix the contents of the tube by vortexing.
4. Mix the 165 kb Ladder aliquot very slowly by pipetting 1-2 times with a wide-bore genomic pipette tip and a pipettor set to ~5 µL volume.
5. Using a regular pipette tip, immediately aliquot 1 µL of the mixed 165 kb Ladder into the tube containing 99 µL of the solution from steps 2 and 3 above (DNF-498 0.25x TE Dilution Buffer + FP-8001 gDNA Diluent Marker). Do not Pipette up-and-down or vortex.
6. Using the wide-bore genomic pipette tip only and a pipettor set to a 20 µL volume slowly pipette the prepared 165 kb Ladder working solution up and down 5 times to mix. This is the 165 kb Ladder Working Solution; use within one day of preparation.
7. Load 20 µL of the 165 kb Ladder Working Solution into Well 12 of a sample plate row that is to be analyzed.
8. The 165 kb Ladder should be run in parallel with the samples for each experiment. It is not recommended to import a previously run 165 kb Ladder.

Sample Plate Preparation

Important gDNA Sampling Procedures:

Before sampling, the sample stock of gDNA must be acclimatized to room temperature for at least 30 minutes.

When mixing large gDNA samples, slowly pipette up-and-down with wide-bore genomic pipette tips.

1. The total input genomic DNA sample concentration should be within a range of 5 pg/µL – 500 pg/µL. If the starting material is higher than 500 pg/µL total concentration, pre-dilute the sample to the specified concentration range with 1x TE buffer. The above genomic DNA sample concentrations assume a starting sample matrix of 1x TE buffer (10 mM Tris- HCl, 1 mM EDTA). If the chloride salt concentration is greater than 10 mM, some loss of sensitivity may be observed, and slight adjustments may need to be made to the sample injection conditions, refer to system user manual for instructions on adjusting injection conditions.
2. Using a clean 96-well sample plate, pipette 18 µL of FP-8001 gDNA Diluent Marker Solution (DM) to each well of the 96-well plate to contain a sample. Fill any unused wells within the row of the sample plate with 20 µL/well of BF-P25 Blank Solution.
3. Pipette 2 µL of each gDNA sample into the 18 µL of DM in the respective wells of the Sample Plate. Mix the sample wells by pipetting up/down 2-3 times with a wide-bore genomic pipette tip and the pipettor set to ~18 µL volume.
4. Load 20 µL of the 165 kb Ladder Working Solution into Well 12 of a sample plate row that is to be analyzed (see previous section).
5. After loading the samples and 165 kb Ladder Working Ladder in each well, check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. Centrifuge the plate to remove any trapped air bubbles. The presence of trapped air bubbles can lead to injection failures.
6. Run the sample plate immediately once prepared, or cover the sample plate with a cover film, store at 2-8°C, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (20 µL/well). The sample plate should be analyzed within a day after preparation.
7. To run the samples, place the plate in one of the three sample plate trays (Drawers 4-6 from the top) of the Femto Pulse instrument. Load the experimental method as described in the following sections.

NOTE: Avoid total DNA input sample concentrations above the specified limits. Overloading of DNA sample can result in saturation of the CCD detector and poor results. The peak heights for individual DNA fragments should lie in an optimal range between 100–5,000 RFUs.

Gel preparation

Prepare gel/dye mixture for Femto Pulse 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, when possible.

Femto Pulse system volume specifications

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of Separation Gel	Volume of 1x Conditioning Solution
12	1.0 µL	10 mL	10 mL
24	2.0 µL	20 mL	20 mL
36	3.0 µL	30 mL	30 mL
48	4.0 µL	40 mL	40 mL
96	8.0 µL	80 mL	80 mL

[1] One sample well per separation is dedicated to the ladder.

Method D Flush

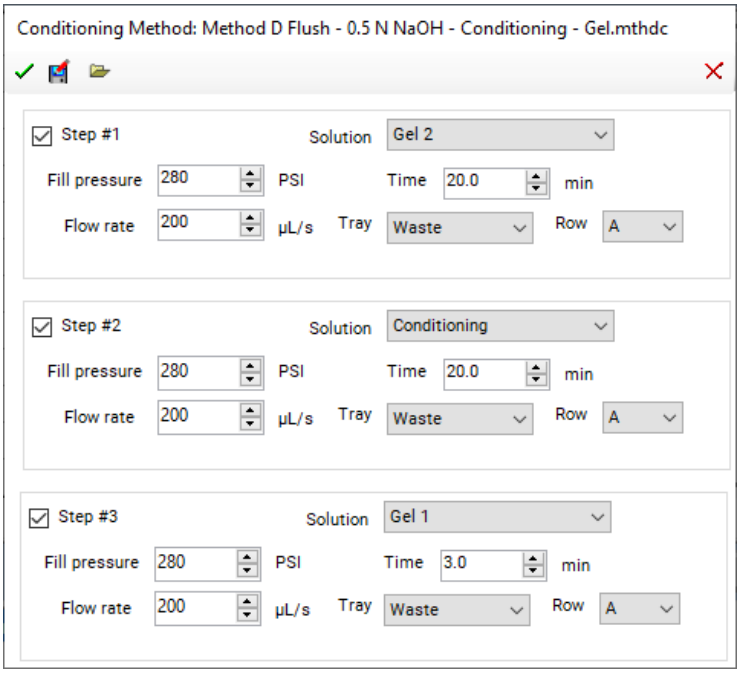
Occasionally when performing separations of high molecular weight (HMW) DNA > 100kb, loss of HMW DNA peak shape or peak signal can occur. In such cases, it is recommended to perform an additional cleaning of the capillary array with 0.5 N NaOH solution, conditioning solution, and gel using the Method D Flush to restore separation performance. Instructions for performing this protocol are outlined below.

WARNING 0.5 N NaOH is corrosive

- Use extreme caution when handling, as exposure can cause severe eye and skin burns. Avoid contact with eyes, skin, or clothing. Wear eye protection and impervious gloves. Clearly label containers to avoid accidental exposure.
- Refer to the product material safety datasheets for all warnings and precautions before proceeding.

1 From the main screen of the Femto Pulse controller software, select the **Operation** tab. Under the **Capillary Array > Conditioning** field click **Add to queue**. The **Select Conditioning Method** form will be displayed, enabling the user to select the conditioning method from the dropdown menu.

2 Select **Method D Flush – 0.5 N NaOH – Conditioning – Gel** from the method dropdown menu. This method will perform a 20 min 0.5 N NaOH solution flush from the Gel2 fluid line, a 20 min conditioning solution flush from the Conditioning line, and a 3 min gel flush from the Gel1 line (figure below).



3 Click **OK** to add the method to the instrument queue (click **Cancel** to abort adding the method).

4 Open the Femto Pulse system side compartment and replace the **Gel2** bottle with a bottle containing a minimum of 25 mL of 0.5N NaOH solution.

5 Ensure there is a minimum of 25 mL of 1x Conditioning Solution in the **Conditioning** bottle, and a minimum of 10 mL of FP Large DNA Separation Gel in the **Gel1** bottle.

6 Close the door to the system side compartment and click the **Play**  icon to start the selected capillary conditioning method.

7 Once the capillary conditioning method is complete, open the waste drawer and remove the 96-deepwell 1mL plate. Empty the waste plate contents in the proper waste disposal area and return the empty plate to the waste drawer.

8 The Femto Pulse system is now ready to run additional samples or can be stored until next use.

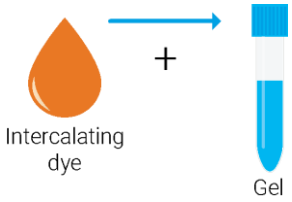
Daily Conditioning (Recommended)

For optimal array performance when running the FP-1002 Genomic DNA 165 kb kit, it is recommended to perform an additional daily conditioning of the capillary array for 20 min.

1. From the main screen of the Femto Pulse control software, select the Operation tab. Under the Capillary Array > Conditioning field press **Add to queue**. The Select Conditioning Method form will be displayed, enabling the user to select the conditioning method from the dropdown menu.
2. Select the "20 min Conditioning" method from the dropdown menu. This method performs a 20 min conditioning solution flush followed by a 3 min Gel fill.
3. Press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).
4. Press the Play icon to start the sequence loaded into the queue.

Agilent FP-1002 Genomic DNA 165 kb 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. Femto Pulse system; Fill row A of buffer plate

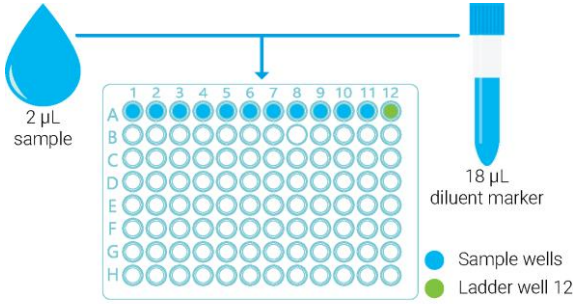
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

    3.1. Femto Pulse system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B "

4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

    4.1. Femto Pulse system; Fill row A of sample plate

5. Mix samples with Diluent Marker in sample plate, add 24 µL of BF-25 Blank Solution to unused wells.
6. Place 20 µL of working ladder solution in well 12 of row to be used.



Femto Pulse system; Ladder – well 12, depending on which row is chosen

WARNING

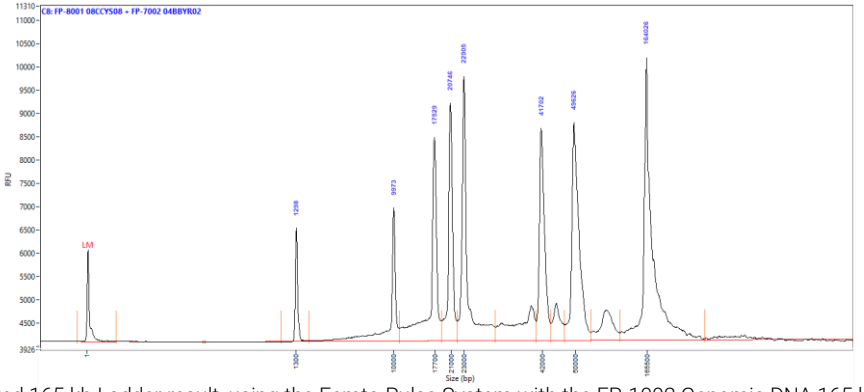
Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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.

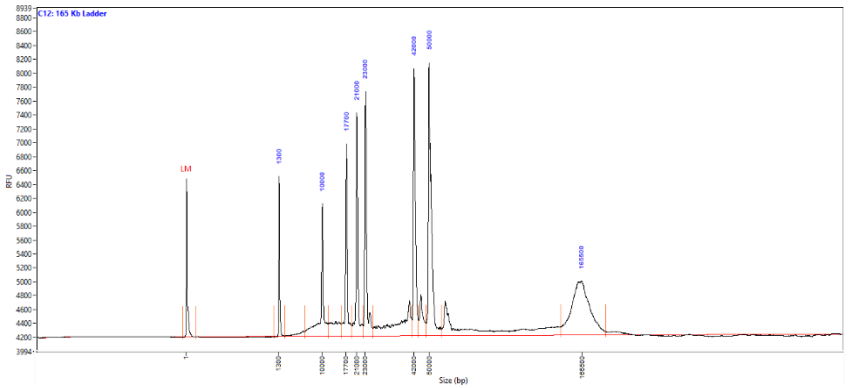
Agilent Femto Pulse software operating procedure

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 FP-1002-22 – gDNA 165kb
   - 3.2 FP-1002E-22 – Extended gDNA 165kb
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select ▶ to start the separation.

165 kb Ladder result



Expected 165 kb Ladder result, using the Femto Pulse System with the FP-1002 Genomic DNA 165 kb kit. Peaks are annotated by size (bp). **Method: FP-1002-22 – gDNA 165kb**.



Expected 165 kb Ladder result, using the Femto Pulse System with the FP-1002 Genomic DNA 165 kb kit. Peaks are annotated by size (bp). Method: **FP-1002E22 – Extended gDNA 165kb**

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the FP-1002 Genomic DNA 165 kb kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
The 165 kb Ladder peak is lower than 1,000 RFUs or non-present.	1 The Femto Pulse capillary array may require a NaOH conditioning flush. If the Method D Flush is not successful, proceed to steps 2 and 3.	1 Perform the Method D Flush. Refer to Appendix A-Method D Flush for detailed instructions.
	2 The 165kb Ladder has degraded, or handling instructions have not been followed.	2 Start with the new aliquot of the 165 kb Ladder. Prepare and handle the Ladder as directed in the manual.
	3 The 165 kb Ladder was vortexed or pipetted with regular pipette tips (not wide-bore tips).	3 Handle the ladder as directed in the manual.
Narrow, high molecular weight peak at the end of the gDNA smear.	1 The gDNA sample is too large for resolution using the fast FP-1002-22-gDNA 165kbmethod.	1 Analyze the gDNA sample using the FP-1002E22-Extended gDNA 165kb method.
The peak signal is >>5,000 RFU.	1 Input sample concentration too high.	1 Further dilute input sample concentration with 1xTE buffer and repeat the experiment OR Reduce injection time and/or injection voltage and repeat the experiment.
No expected gDNA smear or DNA fragment peak observed. Lower Marker observed.	1 Sample concentration is below detection. 2 Sample was not added to a sample plate, or wrong sample row was selected for analysis.	1 Prepare more concentrated sample and repeat the experiment. 2 Verify sample was correctly added or the correct sample row was selected for the analysis.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate and/or marker plate well or bubbles present in well. 2 Insufficient sample volume. A minimum of 20µL is required. 3 Capillary is plugged.	1 Check sample/marker plate wells for trapped air bubbles. Centrifuge the plate. 2 Verify proper volume of solution was added to sample well and marker 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 the Femto Pulse 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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