Agilent Ultra Sensitivity RNA 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 Ultra Sensitivity RNA Kit (275 Samples) (Part # FP-1201-0275) is designed for the sizing and quantitation of total RNA or messenger RNA samples at low sample concentrations.

Specifications

Analytical specifications	Ultra Sensitivity RNA assay – Total RNA
Sizing Range	200 nt – 6000 nt
Sizing Precision	20% CV
RNA Detection Range[1]	2.5 pg/µL - 250 pg/µL input RNA
RNA Quantification Range[1]	15 pg/µL - 250 pg/µL input RNA
RNA Quantification Accuracy[1]	± 30%
RNA Quantification Precision[1]	20% CV
Analytical specifications	**Ultra Sensitivity RNA assay – mRNA**
Sizing Accuracy	± 20%
Sizing Precision	20% CV
RNA Detection Range[2]	15 pg/µL - 500 pg/µL input RNA
RNA Quantification Range[2]	25 pg/µL - 500 pg/µL input RNA
RNA Quantification Accuracy[1]	± 30%
RNA Quantification Precision[1]	20% CV
Physical Specifications	
Total electrophoresis run time	45 minutes
Samples per run	12-Capillary: 11 (+1 Ladder Well)
Sample volume required	2 µL
Kit stability	4 months

[1] Results using FP US RNA Ladder, Universal Mouse Reference total RNA, and Corn Leaf total RNA as samples.
[2] Results based on Mouse Kidney mRNA

Handling Recommendations

- Always thaw FP US RNA Ladder, FP US RNA Diluent Marker, and FP RNA Dilution Buffer on ice and keep them on ice.

- It is highly recommended to aliquot the FP US RNA Ladder prior to first use, to minimize the number of freeze/thaw cycles. Using the provided Eppendorf LoBind 0.5 mL tubes, aliquot 3µL of FP US RNA Ladder per tube into 5 tubes and store the aliquots at -80°C.

- Refer to the product material safety datasheets for safety and handling information.

- RNA samples and RNA ladders are very sensitive to RNase contamination, which can lead to experimental failure. To minimize RNase contamination, wear gloves when working with RNA samples and reagents, and when handling accessories that will come in contact with the RNA sample. Use certified RNase-free plastics and disposable consumables. It is also recommended to work in a separate lab space if possible and decontaminate the pipettes and work surface to avoid cross contamination.

Kit Components – 275 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6606*		Ultra Sensitivity RNA, 275, 4C	
	FP-5201-0250	FP RNA 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-497-0060	0.25x TE Rinse Buffer, 60mL	1
FP-1201-FR*		Ultra Sensitivity RNA, FR	
	FP-6001-U030	FP Intercalating Dye, 30 µL	1
	FP-6501-0003	FP RNA Dilution Buffer, 3 mL	1
	FP-7201-U015	FP US RNA Ladder, 15 µL	1
	FP-8201-0003	FP US RNA Diluent Marker, 3mL	2
5191-6619*		Femto Pulse, RT	
	C27-130	Eppendorf LoBind 0.5 mL Tubes (Bag of 50)	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 systems 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 systems 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)
Steps before sample preparation	• Allow reagents to equilibrate at room temperature for 30 min prior to use, except for the reagents noted above
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. 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.
3. Remove the FP US RNA Ladder from -80°C, and the FP US RNA Diluent Marker (DM) as well as the FP RNA Dilution Buffer (if needed) from -20°C and keep them on ice before use. Vortex the tubes briefly to mix the contents. Spin down the tubes after mixing to ensure liquid is at the bottom of the tube.

FP US RNA Ladder Preparation

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

1. Thaw the RNA ladder on ice, agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing. Using the provided Eppendorf LoBind 0.5 mL tubes, aliquot 3µL of the RNA ladder per tube into 5 tubes and store the aliquots at -80°C.
2. Thaw an RNA ladder aliquot on ice.
3. Heat-denature the entire 3 µL aliquot of the RNA ladder at 70°C for 2 min, immediately cool to 4°C and keep on ice.
4. Transfer 2 µL of denatured RNA ladder to a fresh Lo-Bind tube and add 18 µL of the provided FP RNA Dilution Buffer (FP-6501-0003); mix thoroughly. This is now the working RNA ladder solution.
5. Store any unused portion of the working RNA ladder at -80°C. The working RNA ladder should not need to be heat-denatured again. Each diluted aliquot is good for 5 freeze/thaw cycles.

mRNA Sample Preparation

1. Heat-denature the mRNA samples at 70°C for 2 min if needed and immediately cool to 4°C and keep on ice before use.
2. The input concentration of the mRNA sample MUST be within a range of 15 pg/µL to 500 pg/µL for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with the provided FP RNA Dilution Buffer.

NOTE: Avoid total RNA or mRNA input sample concentrations above the specified limits. Overloading of RNA sample can result in saturation of the CCD detector and poor results. The peak heights for individual RNA fragments in total RNA or mRNA should lie in an optimal range between 10 – 3,000 RFUs.

Sample Plate Preparation

1. Using a clean RNase-free 96-well sample plate, pipette 18 µL of FP US RNA Diluent Marker (DM) solution (FP-8201) to each well in a row that is to contain sample or RNA ladder.
2. Fill any unused wells within the row of the sample plate with 20 µL of BF-P25 Blank Solution.

3. RNA ladder: The RNA ladder must be run in parallel with the samples for each experiment to ensure accurate quantification.

    a) Pipette 2 µL of working RNA ladder Solution (prepared above) into the 18 µL of DM solution in the designated ladder well (Well #12) of each row to be analyzed.

    b) Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

4. Samples: Pipette 2 µL of each DNA sample into the 18 µL of DM Solution in the respective wells of the Sample Plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

5. After mixing sample/RNA ladder and DM solution in the sample plate, 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.

6. For best results, run the plate as soon as possible. If the sample plate will not be used immediately, cover the sample plate with a RNase-free plate seal, store at 2-8°C and use within the same day. Bubbles may develop in the sample wells while sitting at 2-8°C, make sure to centrifuge the plate again and remove the seal before placing the plate into the instrument. 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 System. Load the experimental method as described in the following sections.

Important Sample Mixing Information

When mixing sample with the diluted DM 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 or ladder to the 18 µL of DM Solution, swirl the pipette tip while pipetting up/down to further mix. OR
- After adding 2 µL of sample or ladder to the 18 µL of DM Solution, 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. OR
- After adding 2 µL of sample or ladder to the 18 µL of DM Solution, use a separate pipette tip set to a larger 20 µL volume, and pipette each well up/down to further mix. OR
- 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/mixing.

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.

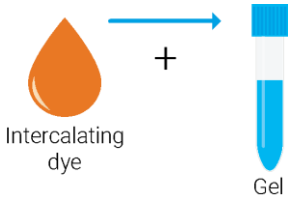
Daily Conditioning (Recommended)

For optimal array performance when running the FP-1201 Ultra Sensitivity RNA 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 controller 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-1201 Ultra Sensitivity RNA 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

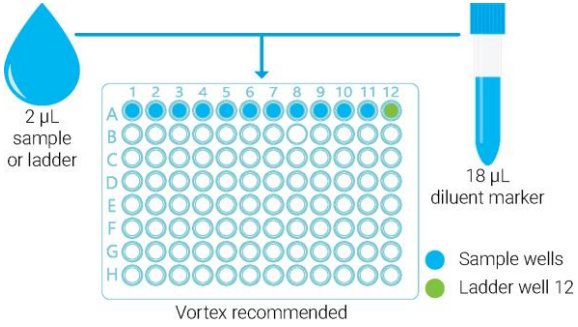
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

    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 or Ladder with Diluent Marker in sample plate, add 24 µL of BF-P25 Blank Solution to unused wells. Place ladder in corresponding well dependent on the capillary size.



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

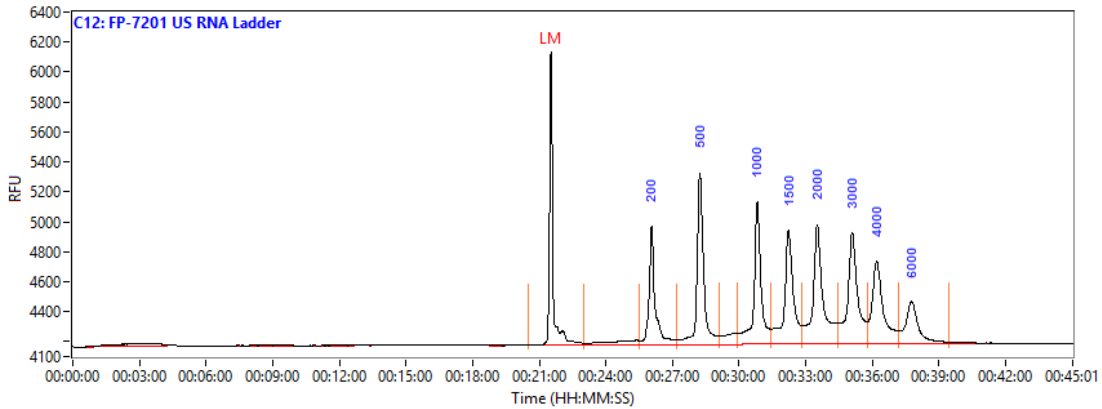
WARNING Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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-1201T22 – US Total RNA
   3.2 FP-1201M22 – US mRNA
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

FP US RNA Ladder result



Representative Ultra Sensitivity RNA Ladder result, using the Femto Pulse System with the FP-1201-US RNA Kit. Peaks are annotated by size (nt). Method: FP-1201T22 -US Total RNA.

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the FP-1201 Ultra Sensitivity RNA Kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
The peak signal is >> 3,000 RFU.	1 Input sample concentration is too high.	1 Dilute input sample with FP RNA Dilution Buffer and repeat experiment; OR Reduce injection time and/or injection voltage, and repeat experiment.
Sample and/or ladder signal too weak or degraded.	1 Sample and/or ladder degraded.	1 Use fresh sample and/or ladder.
	2 Diluent marker degraded.	2 Make sure the diluent marker is stored at 20ºC and keep on ice before use. Use a new vial of diluent marker.
	3 Sample, ladder, diluent buffer, and/or diluent marker are contaminated.	3 Clean working area and equipment with RNaseZap. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot, and diluent marker.
	4 Sample concentration is too low and out of range.	4Verify sample was within concentration range specified for the Ultra Sensitivity RNA kit. Prepare sample at higher concentration; OR Repeat experiment using increased injection time and/or injection voltage.
	5 Sample not added to Diluent Marker solution or not mixed well.	5 Verify sample was correctly added and mixed to sample well.
	6 Rinse buffer is not fresh.	6 Prepare a new rinse buffer plate with 200 µL/well 0.25x TE buffer.
	7 Array was contaminated.	7 Follow Method C outlined in the Appendix – Capillary Array Cleaning of the Femto Pulse User Manual to decontaminate and clean the capillary array.
No peak observed for sample. Lower Marker peak observed.	1 Sample concentration too low and out of range.	1 Prepare more concentrated sample and repeat experiment OR repeat experiment using increased injection time and/or injection voltage for sample plate.
	2 Sample was not added to sample plate.	2 Verify sample was correctly added to the sample plate for the analysis.

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.

www.agilent.com

Edition 02/22

SD-AT000144