# Agilent FP-1201 Ultra Sensitivity RNA

# Quick Guide for the Femto Pulse

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

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 |
| Guaranteed Shelf Life | 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

## 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.

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications. Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

WARNING

**Working with Chemicals**

- **Refer to product safety data sheets for further information**
- **When working with the Fragment Analyzer kit components follow the appropriate safety procedures such as wearing personal protective equipment (PPE).**

## Additional Material Required for Analysis with the Femto Pulse System

| Instrument | Compatible Arrays | Part Number |
|---|---|---|
| Femto Pulse System | FP 12-Capillary Array, 22 cm | A1600-1250-2240 |

### Software

- Fragment Analyzer controller software
- ProSize data analysis software

### Reagents

- Capillary Storage Solution (GP-435-0100)

## 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
- Adhesive PCR plate seal
- Wide-Bore Genomic pipette tips, Thermo Scientific Part #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 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

## Essential Measurement Practices

| | |
|---|---|
| Environmental conditions | • Ambient operating temperature: 19 – 25 °C (66 – 77 °F)<br>• Keep reagents during sample preparation at room temperature |
| Sample Input Concentration | • Ensure sample input concentrations lie within kit specifications.<br>• Sample signal should not exceed 60,000 RFU. |
| 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<br>• Ensure that no sample or Diluent Marker remains within or on the outside of the tip |
| General Information | • 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.<br>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.<br>• 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 use, the RNA ladder 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:
  1. 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
  2. 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
  3. 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
  4. 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.

### Daily Conditioning (Recommended)

- For optimal array performance when running FP-1201 Ultra Sensitivity RNA kit, it is recommended to perform an additional daily conditioning of the capillary array for 20 minutes.
  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.

## Gel Preparation

Centrifuge dye prior to opening the vial to reduce risk of leaking. Ensure the gel + dye is mixed without generating bubbles, gently invert tube 5-10 times.

| Number of Samples | Intercalating Dye Volume (µL) | Separation Gel Volume (mL) |
|---|---|---|
| 12 | 1 | 10 |
| 24 | 2.0 | 20 |
| 36 | 3.0 | 30 |
| 48 | 4.0 | 40 |
| 96 | 8.0 | 80 |

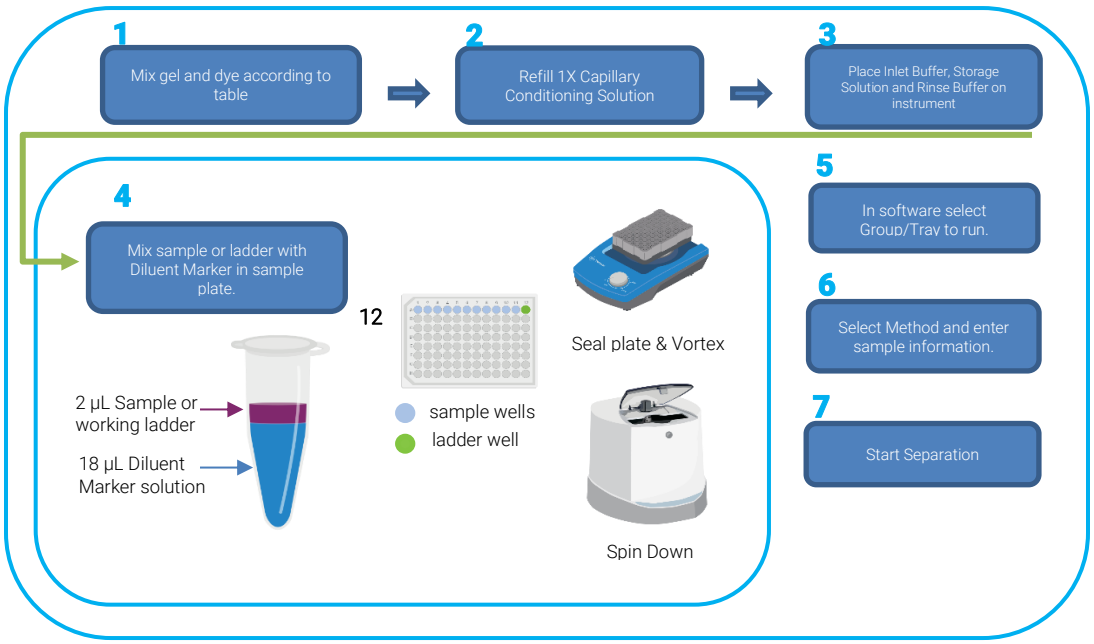
## Conditioning Solution

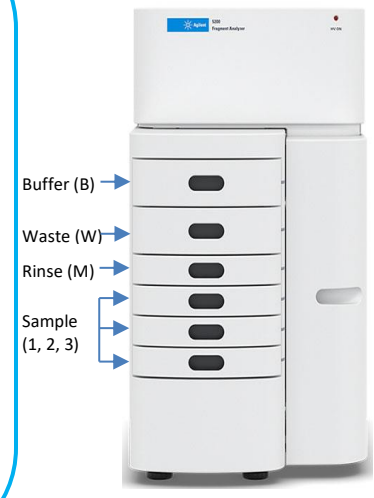
The provided 5X Conditioning Solution must be diluted to 1X using submicron DI water prior to use. Invert to mix.

| Number of Samples | Volume of 1X Conditioning Solution (mL) |
|---|---|
| 12 | 10 |
| 24 | 20 |
| 36 | 30 |
| 48 | 40 |
| 96 | 80 |

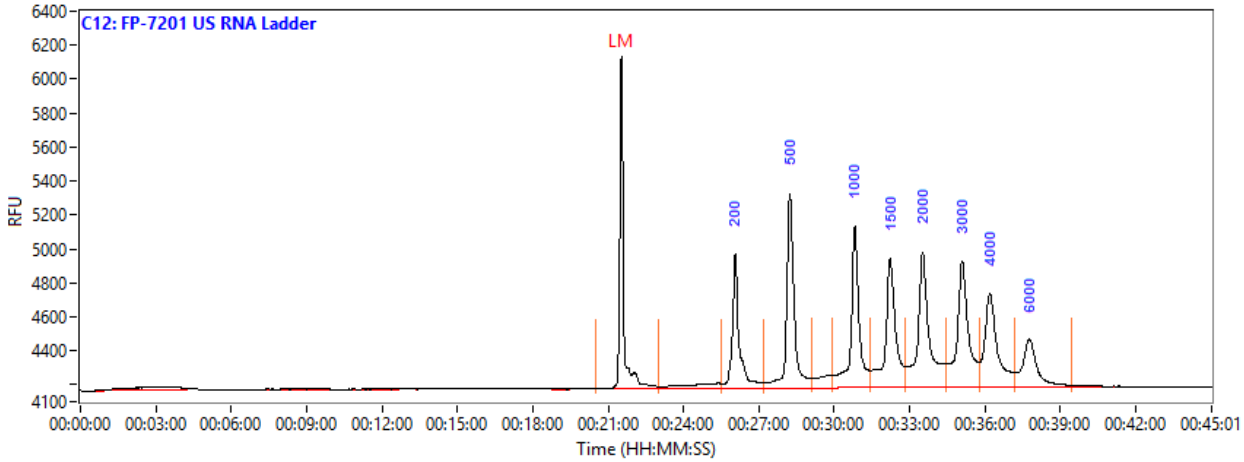
### Agilent FP-1201 Ultra Sensitivity assay operating procedure

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 Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.
   - Femto Pulse system; Fill row A of buffer plate
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.
   - 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.
   - 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.
   - Place ladder in corresponding well 12 (see sample plate image below).
6. Select Row/Group/Tray to run. Enter sample ID and Tray ID, if desired.
7. Add to queue, from the dropdown select the corresponding method based on the sample type and capillary length;
   - FP-1201T22 – US Total RNA
   - FP-1201M22 – US mRNA
8. Add method to the queue by selecting "OK", press play 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 >60,000 RFU. | 1 Input sample concentration is too high. | 1 Dilute input sample with FP RNA Dilution Buffer 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 20°C 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. | 4 Verify sample was within concentration range specified for the Ultra Sensitivity RNA kit. Prepare sample at higher concentration. |
| | 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. |
| | 2 Sample was not added to sample plate. | 2 Verify sample was correctly added to the sample plate for the analysis. |

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Not for use in diagnostic procedures.

**Technical Support and Further Information**
For technical support please visit www.agilent.com which offers useful information and support regarding the products and technology.

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SD-AT000144 Edition 03/25