Agilent HS RNA Kit (15 nt)

Kit Guide

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

 HS RNA kit (15 nt), 500 Samples (Part # DNF-472-0500)
 HS RNA kit (15 nt), 1,000 Samples (Part # DNF-472-1000)
Notices

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The Total RNA – High Sensitivity Assay (50 pg/µL – 5,000 pg/µL input sample concentration).
The mRNA – High Sensitivity Assay (250 pg/µL – 5,000 pg/µL input sample concentration).

Table 1 Physical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume Required</td>
<td>2 µL</td>
</tr>
<tr>
<td>Number of Samples per Run</td>
<td>12-Capillary: 11 (+ 1 well DNA Ladder) or 12 (Imported DNA Ladder)²</td>
</tr>
<tr>
<td></td>
<td>48-Capillary: 47 (+ 1 well DNA Ladder) or 48 (Imported DNA Ladder)²</td>
</tr>
<tr>
<td></td>
<td>96-Capillary: 95 (+ 1 well DNA Ladder) or 96 (Imported DNA Ladder)²</td>
</tr>
<tr>
<td>Total Electrophoresis Run Time</td>
<td>31 min (22-47 Array)</td>
</tr>
<tr>
<td></td>
<td>40 min (33-55 Array)</td>
</tr>
<tr>
<td></td>
<td>70 min (55-80 Array)</td>
</tr>
</tbody>
</table>

Table 2 Analytical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sizing Accuracy¹</td>
<td>± 20%</td>
</tr>
<tr>
<td>Sizing Precision¹</td>
<td>20% CV</td>
</tr>
<tr>
<td>Limit of Detection (S/N &gt; 3)</td>
<td>50 pg/µL</td>
</tr>
<tr>
<td></td>
<td>250 pg/µL</td>
</tr>
<tr>
<td>Quantitative Range (per smear)</td>
<td>50 pg/µL - 5000 pg/µL</td>
</tr>
<tr>
<td></td>
<td>500 pg/µL - 5000 pg/µL</td>
</tr>
<tr>
<td>Quantification Accuracy¹</td>
<td>± 30 %</td>
</tr>
<tr>
<td>Quantification Precision¹</td>
<td>20% CV</td>
</tr>
</tbody>
</table>

¹ Results using RNA Ladder as sample and 33-55cm capillary array
² Results using Total RNA and ribo-depleted mRNA samples diluted in nuclease-free water.
³ The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.
#### Agilent HS RNA Kit (15 nt)

**Table 3  Storage Conditions**

<table>
<thead>
<tr>
<th>Store at −70°C</th>
<th>Store at −20°C:</th>
<th>Store at 2-8°C (Do Not Freeze):</th>
<th>Store at Room Temperature (Do Not Freeze):</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS RNA Ladder*</td>
<td>Intercalating Dye</td>
<td>RNA Separation Gel</td>
<td>5x Capillary Conditioning Solution</td>
</tr>
<tr>
<td>HS RNA Diluent Marker (15 nt)*</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BF-1 Blank Solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25x TE Rinse Buffer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE**
*Always thaw RNA Ladder and RNA Diluent Marker on ice and keep them on ice. Ensure all other reagents are completely warmed to room temperature prior to use.*

**HS RNA kit (15 nt), 500 Samples (Part # DNF-472-0500)**

**Table 4  kit Components**

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNF-265-0240</td>
<td>RNA Separation Gel</td>
<td>240 mL</td>
</tr>
<tr>
<td>DNF-600-U030</td>
<td>Intercalating Dye</td>
<td>30 μL</td>
</tr>
<tr>
<td>DNF-355-0125</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>125 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-475-0050</td>
<td>5x Capillary Conditioning Solution</td>
<td>50 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
</tbody>
</table>
| DNF-370-0004 | HS RNA Diluent Marker (15 nt)  
• Lower Marker in diluent solution (set to 1 bp) | 4 mL x 3 vials |
| DNF-386-U015 | HS RNA Ladder  
• 25 ng/μL | 15 μL, (dilute with RNase-free water prior to use) |
| DNF-497-0250 | 0.25x TE Rinse Buffer | 250 mL |
| DNF-495-0060 | Dilution Buffer 1x TE | 60 mL |
| DNF-301-0008 | BF-1 Blank Solution | 8 mL |
| | Eppendorf LoBind 0.5 mL Tubes | Package of 50 |
Agilent HS RNA Kit (15 nt)

HS RNA kit (15 nt), 1,000 Samples (Part # DNF-472-1000)

Table 5  kit Components

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNF-265-0500</td>
<td>RNA 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-355-0125</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>300 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-475-0050</td>
<td>5x Capillary Conditioning Solution</td>
<td>100 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-370-0004</td>
<td>HS RNA Diluent Marker (15 nt)</td>
<td>4 mL x 6 vials</td>
</tr>
<tr>
<td>DNF-386-U015</td>
<td>HS RNA Ladder</td>
<td>15 μL x 2 (dilute with RNase-free water prior to use)</td>
</tr>
<tr>
<td>DNF-497-0250</td>
<td>0.25x TE Rinse Buffer</td>
<td>250 mL</td>
</tr>
<tr>
<td>DNF-495-0060</td>
<td>Dilution Buffer 1x TE</td>
<td>60 mL</td>
</tr>
<tr>
<td>DNF-301-0008</td>
<td>BF-1 Blank Solution</td>
<td>8 mL</td>
</tr>
<tr>
<td></td>
<td>Eppendorf LoBind 0.5 mL Tubes</td>
<td>Package of 50</td>
</tr>
</tbody>
</table>

**NOTE**
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.

**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.
2 Additional Material and Equipment Required

Material and Equipment Required for Analysis with the Fragment Analyzer

Hardware:
- Fragment Analyzer with LED fluorescence detection:
  - 5200 Fragment Analyzer (Part # M5310AA)
  - 5300 Fragment Analyzer (Part # M5311AA)
  - 5400 Fragment Analyzer (Part # M5312AA)
- FA 12-Capillary Array Ultrashort, 22cm (Part # A2300-1250-2247), OR
- FA 12-Capillary Array Short, 33cm (Part # A2300-1250-3355), OR
- FA 12-Capillary Array Long, 55cm (Part # A2300-1250-5580), OR
- FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355), OR
- FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)

Software:
- Fragment Analyzer control software (Version 1.1.0.11 or higher)
- ProSize data analysis software (Version 2.0.0.61 or higher)

Reagents:
- Capillary Storage Solution, 100 mL (Part #GP-440-0100)
Additional Equipment/Reagents Required (Not Supplied)

- RNase-free water (for diluting sample and HS RNA Ladder)
- Sub-micron filtered DI water system (for dilution of 5x 930 Inlet Buffer and 5x Capillary Conditioning Solutions)
- RNaseZap, Ambion #AM9782 or equivalent product
- Single channel pipettes (for use in 2 µL and 18 µL volumes) and 12-channel pipettes (for use in 20 µL volume) with RNase-free pipette tips
- Additional Eppendorf DNA LoBind tubes, 0.5 mL (Eppendorf #022431005; as needed)
- Thermal cycler (for sample denaturing)
- RNase-free 96-well PCR sample plates. Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list.
- Fisherbrand 96 DeepWell 1mL Plate, Natural Polypropylene, Fisher #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 Separation 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 Separation Gel volume and dilution of 5x 930 Inlet Buffer and 5x Conditioning Solution)
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Vortexer
Gel Preparation

1. Store the RNA 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, the Intercalating Dye should be used at 2x normal concentration (1:10,000 dilution).

3. Bring the RNA Separation Gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and RNA Separation Gel necessary for one day of operation. Use a 50 mL conical centrifuge tube to allow a small minimum working volume. For larger volumes, use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment.
5. The volume of RNA Separation Gel required per run varies between 12-capillary, and 96-capillary Fragment Analyzer systems. The volumes required are summarized below.
### Table 6  Volume Specifications for 12-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of separation gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.0 µL</td>
<td>10 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>24</td>
<td>1.5 µL</td>
<td>15 mL</td>
<td>15 mL</td>
</tr>
<tr>
<td>36</td>
<td>2.0 µL</td>
<td>20 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td>48</td>
<td>2.5 µL</td>
<td>25 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td>96</td>
<td>4.5 µL</td>
<td>45 mL</td>
<td>45 mL</td>
</tr>
</tbody>
</table>

<sup>1</sup> A 5 mL minimum volume should be initially added to the tube. One sample well per separation is dedicated to the ladder.

### Table 7  Volume Specifications for 48-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of separation gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>2.5 µL</td>
<td>25 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td>96</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>144</td>
<td>5.5 µL</td>
<td>55 mL</td>
<td>55 mL</td>
</tr>
<tr>
<td>192</td>
<td>7.0 µL</td>
<td>70 mL</td>
<td>70 mL</td>
</tr>
<tr>
<td>240</td>
<td>8.5 µL</td>
<td>85 mL</td>
<td>85 mL</td>
</tr>
<tr>
<td>288</td>
<td>10.0 µL</td>
<td>100 mL</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

<sup>1</sup> One sample well per separation is dedicated to the ladder.

### Table 8  Volume Specifications for 96-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of separation gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>192</td>
<td>8.0 µL</td>
<td>80 mL</td>
<td>80 mL</td>
</tr>
<tr>
<td>288</td>
<td>12.0 µL</td>
<td>120 mL</td>
<td>120 mL</td>
</tr>
<tr>
<td>384</td>
<td>16.0 µL</td>
<td>160 mL</td>
<td>160 mL</td>
</tr>
<tr>
<td>480</td>
<td>20.0 µL</td>
<td>200 mL</td>
<td>200 mL</td>
</tr>
</tbody>
</table>

<sup>1</sup> One sample well per separation is dedicated to the ladder.

6 Place the RNA 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.

7 When adding RNA Separation Gel to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).

![Solution Levels menu](image)

**Figure 1**  Solution Levels menu

8 When switching applications (e.g., between NGS and RNA kits), prime the appropriate gel fluid line after loading fresh gel/dye mixture. From the main screen of the Fragment Analyzer 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
Agilent HS RNA kit (15 nt) Analysis Protocol

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.
2. In a clean container (e.g. 50 mL or 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. Tables 6-8 show the volume specifications for the conditioning solution.
5. When adding fresh 1x Capillary Conditioning Solution to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).
**Agilent HS RNA kit (15 nt) Analysis Protocol**

**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.
   - 12-Capillary System: Row A only
   - 96-Capillary System: All Rows
   Do not overfill the wells of the inlet buffer plate.

**12-Capillary Systems:**

- In Row H of the same prepared buffer plate, place 1.1 mL/well of Capillary Storage Solution (Part # GP-440-0100).
  Row H of the buffer plate is used for the **Store** location, and the array moves to this position at the end of the experimental sequence.

**96-Capillary Systems:**

- In the Sample 3 drawer, place a sample plate filled with 100 µL/well of Capillary Storage Solution (Part # GP-440-0100).
  Sample 3 is used for the **Store** location, and the array moves to this position at the end of the experimental sequence.

**NOTE**

Ensure Row H of the buffer tray (12-Capillary Systems) or Sample 3 (96-Capillary Systems) is always filled with Capillary Storage Solution, and the capillary array is placed against the Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

3. Place the prepared inlet buffer plate into Drawer "B" (top drawer) of the Fragment Analyzer. 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 Fragment Analyzer. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.

5. Prepare a fresh sample plate filled with 240 µL/well of 0.25x TE Rinse Buffer daily.
   - 12-Capillary System: Row A only
   - 96-Capillary System: All Rows
Place the prepared 0.25x TE Rinse Buffer plate into Drawer “M” (third from top) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.

Ladder and Sample Preparation

General Information

1. The recommended 96-well sample plate for use with the Fragment Analyzer system is a semi-skirted PCR plate from Eppendorf (#951020303). Please refer to Appendix C – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer 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.

2. Remove the HS RNA Diluent Marker (15 nt) from -20°C and keep it on ice before use. Vortex the tube briefly to mix the content. Spin the tube after mixing to ensure liquid is at the bottom of the tube.

HS RNA Ladder Preparation

**NOTE**
Upon arrival of the ladder, it is recommended to divide the ladder into 3 µL aliquots. Store aliquots in the provided Eppendorf LoBind 0.5 mL tubes at -70°C or below.

1. Thaw a 3 µL 25 ng/µL ladder aliquot on ice.
2. Spin down the contents and mix by pipetting the solution up and down with a pipette tip set to a 2 µL volume.
   a. Transfer 2 µL of the 25 ng/µL Ladder to a fresh Eppendorf LoBind 0.5 mL tube.
   **NOTE**
   If more than 2 µL of the 25 ng/µL is transferred for heat-denaturing, be sure to add enough RNase-free water to dilute the ladder to the working concentration of 2 ng/µL.
   b. Heat-denature the ladder at 70°C for 2 min, immediately cool to 4°C and keep on ice.
Agilent HS RNA kit (15 nt) Analysis Protocol

3 Dilute the ladder solution to a working concentration of 2 ng/µL by adding 23 µL of RNase-free water and mixing well. Divide the diluted ladder solution into aliquots with working volume typical for one day use or one sample plate. Store aliquots in the provided Eppendorf LoBind® 0.5 mL tubes at -70°C or below.

Total RNA Sample Preparation

1 Heat-denature the total RNA samples at 70°C for 2 min if needed and immediately cool to 4°C and keep on ice before use.

2 The total RNA input sample must be within a total concentration range of 50 pg/µL to 5000 pg/µL for optimal assay results. If the concentration of the sample is above this range, dilute with RNase-free water.

mRNA Sample Preparation

1 Heat-denature the RNA samples at 70°C for 2 min if needed and immediately cool to 4°C and keep on ice before use.

2 The mRNA input sample must be within a total concentration range of 250 pg/µL to 5000 pg/µL for optimal assay results. If the concentration of the sample is above this range, dilute with RNase-free water.

Sample Plate Preparation

1 The total input RNA sample concentration MUST be within a range of 50 pg/µL to 5000 pg/µL (total RNA) or 250 pg/µL to 5000 pg/µL (mRNA) for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with RNase-free water prior to performing the assay.

2 The above RNA sample concentrations assume the sample is in water. If salt is present, some loss of sensitivity may be observed and slight adjustments may need to be made to the sample injection conditions.

NOTE

Avoid total input RNA 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 RNA smears should lie in an optimal range between 20 – 2000 RFUs. The peak heights for individual RNA fragments in total RNA should lie in an optimal range between 100 – 20,000 RFUs.

3 Using a fresh RNase-free 96-well sample plate, pipette 18 µL of the HS RNA Diluent Marker (15 nt) (DM) Solution to each well in a row that is to contain sample or RNA Ladder. Fill any unused wells within the row of the sample plate with 20 µL/well of BF-1 Blank Solution.
4 Pipette 2 µL of each denatured RNA sample into the respective wells of the sample; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

5 RNA Ladder: The RNA Ladder must be run in parallel with the samples for each experiment to ensure the accurate quantification. Thaw the denatured 2 ng/µL working concentration RNA Ladder on ice. Pipette 2 µL of denatured RNA Ladder into the 18 µL of Diluent Marker (15 nt) (DM) Solution in the designated ladder well:
   - 12-Capillary System: Well 12 of each row to be analysed
   - 96-Capillary System: Well H12

6 Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip or use one of the mixing methods suggested in the following.

NOTE Important Sample Mixing Information:
When mixing sample with diluent marker 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 diluent marker, swirl the pipette tip while pipetting up/down to further mix.
- After adding 2 µL of sample or ladder to the 18 µL of diluent marker, 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 or ladder to the 18 µL of diluent marker, 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.
Agilent HS RNA kit (15 nt) Analysis Protocol

7 After mixing sample/RNA Ladder and Diluent Marker (15 nt) 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.

8 For best results, run the plate as soon as possible. If the sample plate will not be used immediately, cover the sample plate with RNase-free cover film, store at 4°C and use within the same day. Spin the plate again if any bubbles developed in the sample wells. Be sure to remove the cover film before placing the plate into the instrument.

9 To run the samples:
   • In the 12-Capillary System, place the plate in one of the three sample plate trays (Drawers 4-6 from the top).
   • In the 96-Capillary System, place the plate in one of the two available sample plate trays (Drawers 4-5 from the top).

10 Load or create the experimental method as described in the following sections.
Performing Experiments

Running an Experiment

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

   **NOTE**
   For 96-Capillary Systems: Sample 3 is typically assigned to the Capillary Storage Solution.

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](image-url)
After sample information for the row or plate has been entered, under the **Run Selected Group** 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).

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

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., 22cm, 33cm or 55cm). Select the following method:

- **a** Select **DNF-472M22 - HS mRNA 15nt.mthds** when the 22 cm effective, 47 cm total “ultra-short” capillary array is installed (for mRNA).
- **b** Select **DNF-472T22 - HS Total RNA 15nt.mthds** when the 22 cm effective, 47 cm total “ultra-short” capillary array is installed (for Total RNA).
- **c** Select **DNF-472M33 - HS mRNA 15nt.mthds** when the 33 cm effective, 55 cm total “short” capillary array is installed (for mRNA).
- **d** Select **DNF-472T33 - HS Total RNA 15nt.mthds** when the 33 cm effective, 55 cm total “short” capillary array is installed (for Total RNA).
- **e** Select **DNF-472M55 - HS mRNA 15nt.mthds** when the 55 cm effective, 80 cm total “long” capillary array is installed (for mRNA).
- **f** Select **DNF-472T55 - HS Total RNA 15nt.mthds** when the 55 cm effective, 80 cm total “long” capillary array is installed (for Total RNA).

Select the appropriate **Gel** line being used for the experiment (Gel 1 or Gel 2) using the dropdown.
**Agilent HS RNA kit (15 nt) Analysis Protocol**

6 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).

7 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.

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

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

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

11 On 96-Capillary Systems, or in 12-Capillary Systems if the entire 96-well sample tray is to be run using the same experimental method, under the **Run Entire Tray** field press **Add to queue**. A form similar to Figure 4 will be displayed for entering information and adding the run to the instrument queue for the entire 96-well sample tray.

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

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

14 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.
15 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.

16 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.

17 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 Fragment Analyzer User Manual.

18 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 instrument stage will automatically move to the Store location (12-Capillary Systems: Row H of the inlet buffer tray containing the Capillary Storage Solution; 96-Capillary Systems: Sample 3 location).
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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 DNF-472M22 (Figure 6), DNF-472T22 (Figure 7), DNF-472M33 (Figure 8), DNF-472T33 (Figure 9), DNF-472M55 (Figure 10), and DNF-472T55 (Figure 11) methods are shown below. The general steps of the methods are as follows:
   a Full Condition flushing method (Automatically enabled). Default Gel Selection: Gel 1.
   b Perform Prerun (enabled) (7-12 kV, 30 sec)
   c Rinse (DISABLED)
   d Marker Injection (disabled)
   e Rinse (enabled; Tray = Marker; Row = A; # Dips = 2). This step moves to the Marker tray and rinses the capillary tips twice with 0.25x TE Rinse Buffer.
   f Sample Injection (enabled) Voltage Injection (6-12 kV, 150-200 sec). This step injects the prepared sample plate.
   g Separation (enabled) Voltage (7-12 kV, 31-70 min). This step performs the CE Separation.
Figure 6. DNF-472M22 (mRNA) method
Figure 7. DNF-472T22 (tRNA) method
Figure 8. DNF-472M33 (mRNA) method
Figure 9. DNF-472T33 (tRNA) method
Figure 10. DNF-472M55 (mRNA) method
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Figure 11. DNF-472T55 (tRNA) method
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4 An Administrator level user has the option to adjust the Gel Selection; Prerun settings; Rinse settings including Tray, Row and # Dips; Marker Injection settings including Row; Sample Injection settings; and the Separation settings. For example, if the rinse buffer is loaded into a row other than Row A this can be adjusted prior to or while the method is loaded on the experimental queue.

5 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.

Processing Experimental Data

1 When processing data, the ProSize data analysis software will automatically recognize the separation method performed and apply the appropriate matching configuration file from the C:\ProSize 3.0\Configurations directory:

   a The DNF-472M22 separation method will be processed using the DNF-472M22 - HS mRNA (15nt) configuration file;

   b The DNF-472T22 separation method will be processed using the DNF-472T22 - HS Total RNA (15nt) configuration file;

   c The DNF-472M33 separation method will be processed using the DNF-472M33 - HS mRNA (15nt) configuration file;

   d The DNF-472T33 separation method will be processed using the DNF-472T33 - HS Total RNA (15nt) configuration file;

   e The DNF-472M55 separation method will be processed using the DNF-472M55 - HS mRNA (15nt) configuration file;

   f The DNF-472T55 separation method will be processed using the DNF-472T55 - HS Total RNA (15nt) configuration file.

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

The Quantification setting for the ladder Final Conc. (ng/μL) should be set to 0.2 to reflect the higher 2 ng/μL working concentration RNA Ladder. Refer to Product Bulletin PB-2015-002 for instructions on changing the ladder concentration value in ProSize software, or contact Agilent Technical Support.
Agilent HS RNA kit (15 nt) Analysis Protocol

2 The data should be normalized to the lower marker (set to 15 nt), and the size and quantification calibrated to calibrated to the RNA Ladder run in parallel to the samples. Figure 12 shows the typical result for the HS RNA Ladder. A total of 9 peaks should be observed.

3 Start with the preloaded Global Configuration and modify the parameters as needed to fit the data during data processing.

**NOTE**

Note that 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 additional information on processing data, refer to the ProSize User Manual.
Agilent HS RNA kit (15 nt) Analysis Protocol

Fragment Analyzer Shut Down/Storage

Instrument Shut Down/Storage

After each experiment, the instrument automatically places the capillary array in the Store position against Capillary Storage Solution:

- 12-Capillary Systems: Row H of the buffer tray
- 48-Capillary Systems: Sample 3
- 96-Capillary Systems: Sample 3

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.
Checking Your Separation Results

Total RNA Sample

Figure 12 shows the typical result for the HS RNA Ladder from a 33 cm effective, 55 cm total "short" capillary array. The initial concentration of the ladder is 2 ng/μL (final concentration of the ladder after mixing with DM is 0.2 ng/μL). A total of 9 peaks should be observed with the sizes annotated as in Figure 12. The first peak corresponds to the 15 nt lower marker peak (LM).

The size calibration of the HS RNA Ladder should be fitted with a point-to-point curve fitting algorithm in the ProSize 2.0 software. The HS RNA Ladder is run in parallel to the samples for use in calculating the size and concentration of the samples.

Figure 12. Representative HS RNA Ladder result using Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt).
Checking Your Separation Results

Figure 13 shows the typical results for a chicken spleen total RNA sample from a 33 cm effective, 55 cm total “short” capillary array. The data was normalized to the lower marker and the size was calibrated to the HS RNA Ladder run in parallel to the sample.

![Figure 13. Chicken spleen total RNA sample result using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt).](image)

The RNA Property Summary is displayed for each total RNA sample when in the Total RNA analysis mode. This includes the total RNA concentration, the 28S/18S ratio (Eukaryotic mode), and the RNA Quality Number (RQN) (Figure 14).

![Figure 14. RNA Property Summary (Total RNA analysis mode)](image)
Checking Your Separation Results

mRNA Sample

Figure 15 shows the typical result for the HS RNA Ladder using the method for mRNA from a 33 cm effective, 55 cm total “short” capillary array. The initial concentration of the ladder was 2 ng/μL (final concentration of the ladder after mixing with DM was 0.2 ng/μL). A total of 9 peaks should be observed with the sizes annotated as in Figure 15. The first peak was the 15 nt lower marker peak (LM). The size calibration of the HS RNA Ladder should be fitted with a point-to-point curve fitting algorithm in the ProSize 2.0 software.

Figure 15. Representative HS RNA Ladder result using Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt) – mRNA assay
Checking Your Separation Results

Figure 16 shows the typical results for a rat kidney mRNA sample from a 33 cm effective, 55 cm total "short" capillary array. The data was normalized to the lower marker and the size was calibrated to the HS RNA Ladder run in parallel to the sample.

![Figure 16. Rat kidney mRNA sample result using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt) – mRNA assay.](image)

The mRNA Property Summary for each mRNA sample is displayed when in the mRNA analysis mode and reports the % rRNA Contamination (% of ribosomal RNA in the total concentration) (Figure 17).

![Figure 17. mRNA Property Summary (mRNA analysis mode)](image)
5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-472 HS RNA kit (15 nt) and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Table 9 Troubleshooting actions for assay specific issues

<table>
<thead>
<tr>
<th>Issue</th>
<th>Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
</table>
| Sample and/or ladder signal too weak or degraded. | 1 Sample and/or ladder degraded.  
2 Diluent marker degraded.  
3 Sample, ladder and/or diluent marker are contaminated.  
4 Sample concentration is too low and out of range.  
5 Sample not added to Diluent Marker solution or not mixed well.  
6 Rinse buffer is not fresh or a wrong rinse buffer is used.  
7 Array was contaminated. | 1 Use fresh sample and/or ladder.  
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 Clean working area and equipment with RNaseZap. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot, and diluent marker.  
4 Verify sample was within concentration range specified for the HS RNA kit (15 nt). Prepare sample at higher concentration; OR Repeat experiment using increased injection time and/or injection voltage.  
5 Verify sample was correctly added and mixed to sample well.  
6 Prepare a new rinse buffer plate with 240 µL/well 0.25xTE buffer.  
7 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual for details). |
| Sample signal drops abruptly at the end of separation. | 1 Sample concentration too high and out of range. | 1 Verify sample was within concentration range specified for the HS RNA kit (15 nt). |
| Missing 25S or 28S ribosomal peak; missing 6000 nt fragment in ladder. | 1 No rinse buffer in Marker plate row A; wrong rinse buffer.  
2 Dirty array inlet.  
3 Aging array. | 1 Use a fresh rinse buffer plate with 240 µL/well 0.25xTE buffer.  
2 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual for details).  
3 Replace the array with a new array. If issue persists, contact Agilent Technical Support. |
| Split RNA peak. | 1 Sample’s salt concentration was too high. | 1 Take steps to lower the salt content in the sample and repeat experiment. |
Troubleshooting

<table>
<thead>
<tr>
<th>Issue</th>
<th>Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak too broad, signal too low and/or migration time too long.</td>
<td>1 Capillary array needs to be reconditioned.</td>
<td>1 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual for details).</td>
</tr>
<tr>
<td></td>
<td>2 Capillary array vent valve is clogged.</td>
<td>2 Clean vent valve with deionized water (See Fragment Analyzer User Manual for details).</td>
</tr>
<tr>
<td>No sample peak or marker peak observed for individual sample.</td>
<td>1 Air trapped at the bottom of sample plate well, or bubbles present in sample well.</td>
<td>1 Check sample plate wells for trapped air bubbles. Centrifuge plate.</td>
</tr>
<tr>
<td></td>
<td>2 Insufficient sample volume. A minimum of 20 µL is required.</td>
<td>2 Verify proper volume of solution was added to sample well.</td>
</tr>
<tr>
<td></td>
<td>3 Capillary is plugged.</td>
<td>3 Check waste plate for liquid in the capillary well. If no liquid is observed, follow the steps outlined in Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.</td>
</tr>
</tbody>
</table>
In This Book

This kit Guide describes the following:

- Agilent HS RNA Kit (15 nt)
- Additional Material and Equipment Required
- Agilent HS RNA Kit (15 nt) Protocol
- Checking Your Separation Results
- Troubleshooting