# Agilent DNF-472 (15 nt) HS RNA Kit IVT mRNA

# Quick Guide

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

This Quick Guide is intended for use with the Agilent 5200, 5300, and 5400 Fragment Analyzer systems only. The DNF-472 HS RNA kit has methods for analysis of IVT mRNA as well as Total RNA and ribo-depleted RNA samples. This quick guide covers the two methods for IVT mRNA a low concentration range of 500 to 2,500 pg/µL and a mid-concentration range of 2,500 to 10,000 pg/µL, as well as the corresponding Extended methods for IVT mRNA > 6,000 nt. A separate quick guide (SD-AT000132) covers Total RNA and ribo-depleted RNA methods.

## Specifications

| Analytical Specifications[1] | HS RNA (15 nt) Kit | |
|---|---|---|
| Method | Low Concentration<br>DNF-472A33 – HS IVT mRNA Low Concentration | Mid Concentration<br>DNF-472B33 – HS IVT mRNA Mid Concentration |
| Sizing Range | 200 nt – 6,000 nt | 200 nt – 6,000 nt |
| Sizing Accuracy[1] | ±15% | ±15% |
| Size Precision[1] | <10% CV | <5% CV |
| Sensitivity [1] | 15 pg/µL | N/A |
| Input Concentration Rage[1] | 500 pg/µL -2,500 pg/µL | 2,500 pg/µL -10,000 pg/µL |
| Extended Method | Low Concentration<br>DNF-472AE33-HS IVT mRNA Extended Low Concentration | Mid Concentration<br>DNF-472BE33 – HS IVT mRNA Extended Mid Concentration |
| Sizing Range | 500 nt – 9,000 nt<br>with Lonza RNA Marker | 500 nt – 9,000 nt<br>with Lonza RNA Marker |
| Sizing Accuracy[1] | ±15% | ±15% |
| Size Precision[1] | <10% CV | <5% CV |
| Sensitivity[1] | 15 pg/ µL | N/A |
| Input Concentration Rage[1] | 500 pg/µL -2,500 pg/µL | 2,500 pg/µL -10,000 pg/µL |
| **Physical Specifications** | | |
| Total Electrophoresis Run Time | 33cm: 45 minutes<br>33cm: 90 minutes Extended Method | |

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| Samples Per Run | 12, 48 or 96; depending on the instrument type |
| Sample Volume Required | 2 µL |
| Kit Stability | 4 months |

[1] Results using 1,800 nt, 4,300 nt, and 9,000 nt IVT mRNA with no modifications on the 5200 and 5300 Fragment Analyzer instruments with the FA Capillary Array Short, 33 cm.

## Kit Components – 500 Sample Kit – *Refer to product label for proper storage conditions*

| Kit Component Number | Part Number (Re-order Number) | Description | Quantity Per Kit |
|---|---|---|---|
| 5191-6574* | | HS RNA (15 nt), 500, 4 °C | |
| | DNF-265-0240 | RNA Separation Gel, 240 mL | 1 |
| | DNF-301-0008 | BF-1 Blank Solution, 8 mL | 1 |
| | DNF-355-0125 | 5x 930 dsDNA Inlet Buffer, 125 mL | 1 |
| | DNF-497-0250 | 0.25x TE Rinse Buffer, 250 mL | 1 |
| DNF-472-FR* | | HS RNA (15 nt) FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 1 |
| | DNF-370-0004 | HS RNA Diluent Marker (15 nt), 4 mL | 3 |
| | DNF-386-U015 | HS RNA Ladder, 15 µL | 1 |
| 5191-6612* | | Quantitative DNA, RT | |
| | C275-130 | Eppendorf LoBind 0.5 mL tubes (bag of 50) | 1 |
| | DNF-475-0050 | 5x Capillary Conditioning Solution, 50 mL | 1 |

## Kit Components – 1000 Sample Kit – *Refer to product label for proper storage conditions*

| Kit Component Number | Part Number (Re-order Number) | Description | Quantity Per Kit |
|---|---|---|---|
| 5191-6575* | | HS RNA (15 nt), 1000, 4 °C | |
| | DNF-265-0500 | RNA Separation Gel, 500 mL | 1 |
| | DNF-301-0008 | BF-1 Blank Solution, 8 mL | 1 |
| | DNF-355-0300 | 5x 930 dsDNA Inlet Buffer, 300 mL | 1 |
| | DNF-497-0250 | 0.25x TE Rinse Buffer, 250 mL | 1 |
| DNF-472-FR* | | HS RNA (15 nt), FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 2 |
| | DNF-370-0004 | HS RNA Diluent Marker (15 nt), 4 mL | 6 |
| | DNF-386-U015 | HS RNA Ladder, 15 µL | 2 |
| 5191-6613* | | HS RNA (15 nt), 1000, RT | |
| | C275-130 | Eppendorf LoBind 0.5 mL tubes (bag of 50) | 1 |
| | DNF-475-0100 | 5x Capillary Conditioning Solution, 100 mL | 1 |

*Not orderable.

## Additional Material Required for Analysis with Fragment Analyzer Systems (not supplied)

| Instrument | Compatible Arrays | Part Number |
|---|---|---|
| 5200 Fragment Analyzer | FA 12 Capillary Array Short | A2300-1250-3355 |
| 5300 Fragment Analyzer. | FA 48 Capillary Array Short<br>FA/ZAG 96 Capillary Array Short | A2300-4850-3355<br>A2300-9650-3355 |
| 5400 Fragment Analyzer | FA/ZAG 96 Capillary Array Short | A2300-9650-3355 |

### Software

- Fragment Analyzer controller software
- ProSize data analysis software

### Reagents

- Capillary Storage Solution (GP-440-0100)

## Additional equipment required (not supplied)

- 96-well PCR samples plates (*Refer to Appendix in Fragment Analyzer User Manual*)
- Multichannel pipettor and/or liquid handling device capable of dispensing 1-100 µL (sample plates) and 1,000 µL (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge
- Adhesive PCR plate seals
- Sub-micron filtered DI water system: for instrument reagent dilutions
- 96-deepwell 1 mL plate: inlet buffer and/or waste plate (Agilent #P60-20 or Fisher Scientific #12-566-120)
- Reagent reservoir 50 mL: for use in pipetting inlet buffer plates (VWR #89094-680, or similar)
- Conical centrifuge tubes for prepared separation gel+dye mixture and/or 1x Capillary Conditioning Solution
    - 50 mL for 5200 Fragment Analyzer system (BD Falcon #352070, Fisher Scientific #14-432-22 or VWR #21008-940)
    - 250 mL for 5300 and 5400 Fragment Analyzer systems (Corning #430776, Fisher Scientific #05-538-53 or VWR #21008-771)
- Vortexer (VWR, part number 102093-352)
- RNase-free/nuclease free water RNA sample dilutions
- RNaseZap (Ambion #AM9782 or equivalent product)
- Thermal cycler
- Lonza RNA Marker when running Extended IVT mRNA method (Lonza p/n 50705)
- Eppendorf DNA LoBind® tubes for aliquoting RNA Ladder (Eppendorf p/n 022431021)

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).**

## Essential Measurement Practices

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| **Environmental conditions** | • Ambient operating temperature: 19 – 25 °C (66 – 77 °F)<br>• Keep instrument reagents at room temperature during sample preparation. |
| **Sample Input Concentration** | • Ensure sample input concentrations lie within kit specifications.<br>• Sample signal should not exceed 60,000 RFU. |
| **Working with RNA** | • Ensure all working areas, reagents and plastic ware are RNase free.<br>• Handle RNA samples with care.<br>• Wear gloves at all times.<br>• Thaw RNA samples on ice.<br>• Vortex and centrifuge all samples before use.<br>• Store RNA samples on ice throughout the preparation. |
| **Steps before sample preparation** | • Allow instrument reagents to equilibrate at room temperature for 30 min prior to use.<br>• Thaw HS RNA Ladder on ice prior to use.<br>• Keep HS RNA Diluent Marker on ice and in the dark throughout use. Vortex the tube briefly to mix the contents. |
| **Pipetting practice** | • Pipette reagents against the side of the 96-well sample plate or sample tube.<br>• Ensure no sample or Diluent Marker remains within or on the outside of the tip. |
| **HS RNA Ladder Preparation** | • The HS RNA Ladder is supplied at a concentration of 25 ng/µL. Upon arrival of the ladder it is recommended to divide the ladder into 3 µL aliquots. Store aliquots in the provided Eppendorf 0.5 mL LoBind tubes at -70°C or below.<br>• Thaw a 3 µL 25 ng/µL ladder aliquot on ice.<br>• Spin down the contents and mix by pipetting the solution up and down with a pipette tip set to a 2 µL volume.<br>1. Transfer 2 µL of the 25 ng/µL Ladder to a fresh Eppendorf LoBind 0.5 mL tube.<br>2. Heat denature the ladder at 70°C for 2 minutes, immediately cool to 4°C and keep on ice.<br>• 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 a 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.<br>• 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.<br>• The 2 ng/µL working concentration HS RNA Ladder must be run in parallel with the samples for each experiment to ensure accurate sizing. |
| **Extended Method Lonza RNA Marker (Lonza ladder) Preparation** | • Use the Extended Method for analysis of IVT mRNA > 6,000 nt this was developed in conjunction with the Lonza RNA Marker (Lonza ladder), diluted in nuclease free water to a concentration of 2 ng/µL.<br>• Upon arrival of the Lonza ladder dilute to 25 ng/µL with nuclease free water and divide the ladder into 3 µL aliquots. Store aliquots in the provided Eppendorf 0.5 mL LoBind tubes at -70°C or below.<br>• Thaw a 3 µL 25 ng/µL Lonza ladder aliquot on ice.<br>• Spin down the contents and mix by pipetting the solution up and down with a pipette tip set to a 2 µL volume.<br>1. Transfer 2 µL of the 25 ng/µL Lonza ladder to a fresh Eppendorf LoBind 0.5 mL tube.<br>2. Heat denature the Lonza ladder at 70°C for 2 minutes, immediately cool to 4°C and keep on ice.<br>• Dilute the heat denatured Lonza 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 a 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.<br>• If more than 2 µL of the 25 ng/µL ladder 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.<br>• The 2 ng/µL working concentration Lonza ladder must be run in parallel with the samples for each experiment to ensure accurate sizing. |
| **IVT mRNA Sample Preparation** | • Heat-denature the IVT mRNA samples at 70°C for 2 minutes if needed and immediately cool to 4°C and keep on ice before use.<br>• The IVT mRNA input sample must be within a total concentration range of **500 to 2,500 pg/µL** |

| | |
|---|---|
| | for Low Concentration range or **2,500 to 10,000 pg/µL** for Mid Concentration range to achieve optimal kit results. If the concentration of the sample is above this range, dilute with RNase-free water. |
| Sample Plate Preparation | • Using a fresh RNase-free 96-well sample plate, pipette 18 µL of the HS RNA Diluent Marker (DM) solution to each well in a row that is to contain sample or RNA Ladder.<br>• Fill any unused wells within the row of the sample plate with 20 µL of blank solution.<br>• Pipette 2 µL of heat denatured RNA sample into the respective wells of the sample plate.<br>• Thaw the denatured 2 ng/µL working concentration of the RNA Ladder on ice.<br>• Pipette 2 µL of working concentration denatured RNA Ladder into the 18 µL of DM solution in the designated ladder well.<br>• Input range of concentration should not exceed 60,000 RFU<br>• |
| Mixing and centrifugation recommendations | • When mixing sample with DM, mix the contents of the well thoroughly**.** It is suggested to perform ***one*** of the following methods to ensure complete mixing:<br>· After adding 2 µL of sample or ladder to the 18 µL of DM, place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm for 2 min. Avoid intense vortexing that causes splashing. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells.<br>· After adding 2 µL of sample or ladder to the 18 µL of DM, use a separate pipette tip set to a larger 18 µL volume, and pipette each well up/down to further mix.<br>· Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample or ladder volume.<br>• After mixing, centrifuge the plate to remove any air bubbles.<br>• Run samples immediately after preparation. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate. |

## 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 | 1.5 | 15 |
| 48 | 2.5 | 25 |
| 96 | 4.5 | 45 |
| 192 | 8 | 80 |
| 384 | 16 | 160 |

## Conditioning Solution

The provided 5XConditioning Solution must be diluted to 1X using submicron DI water prior to use. Gently invert to mix.

| Number of Samples | Volume of 1X Conditioning Solution (mL) |
|---|---|
| 12 | 10 |
| 24 | 15 |
| 48 | 25 |
| 96 | 45 |
| 192 | 80 |
| 384 | 160 |

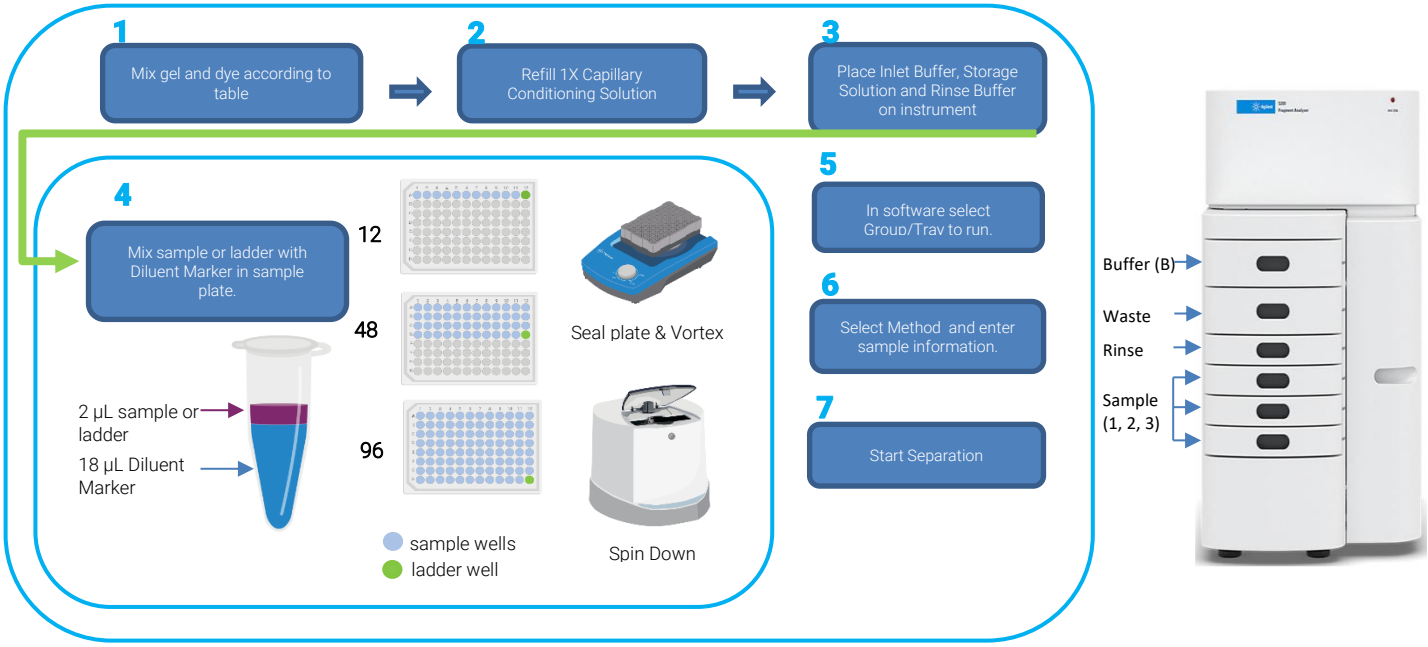
## Agilent HS RNA DNF-472 Kit Operating Procedure

1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1X Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer 'W".
4. Place fresh 1X Inlet Buffer, 1 mL/well, in drawer "B". Replace daily.
   - 5200 – row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows

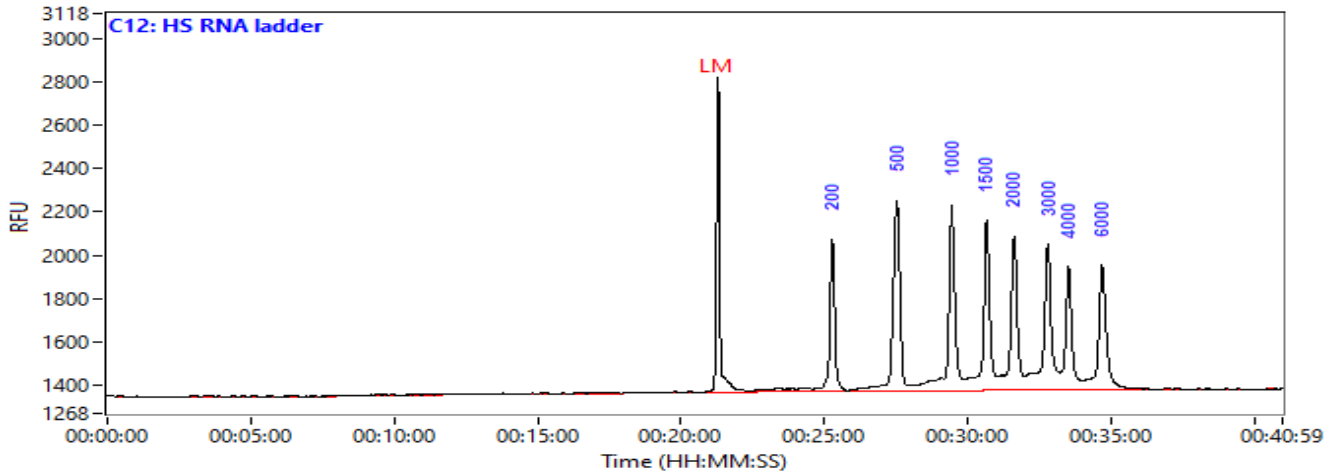
   Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.
   - 5200 – row H, 1 mL/well, drawer B
   - 5300 – 48 capillary, rows A-D, 100 µL/well, drawer 3
   - 5300/5400 – 96 capillary, all rows, 100 µL/well, drawer 3

   Place fresh 0.25x TE Rinse Buffer plate, 200 µL/well, in drawer "M". Replace daily.
   - 5200 – Row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows
5. Mix samples and ladder with diluent marker in sample plate, add 20 µL of Blank Solution to unused wells. Place ladder in corresponding well (see sample plate image below), depending on capillary array used. Use Lonza RNA Marker for Extended Methods.
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;
   - DNF-472A33 – HS IVT mRNA Low Concentration
   - DNF-472AE33 – HS IVT mRNA Extended Low Concentration
   - DNF-472B33 – HS IVT mRNA Mid Concentration
   - DNF-472BE33 – HS IVT mRNA Extended Mid Concentration

   Enter Tray Name, Folder Prefix and Notes, if desired.
8. Add method to the queue by selecting "OK", press play to start the separation.



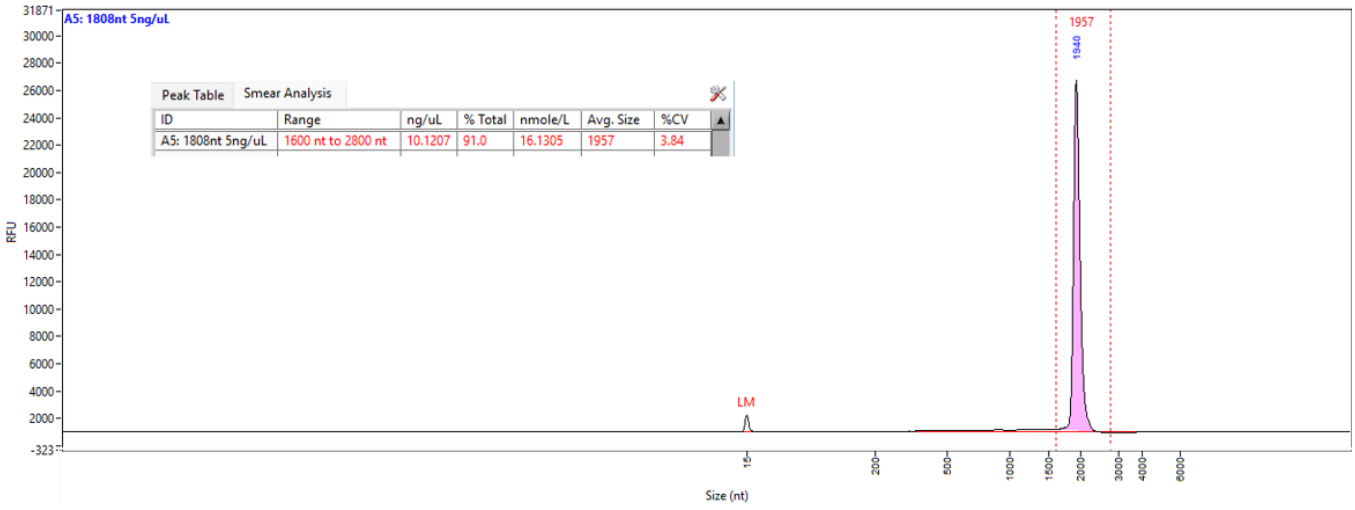
## HS RNA Ladder result




Representative HS RNA Ladder result using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt). Method: DNF-472B33 - HS IVT mRNA Mid Concentration. Peaks annotated by size (nt). RFU values may differ between instruments.

## IVT mRNA Sample: Smear analysis table using normal separation method

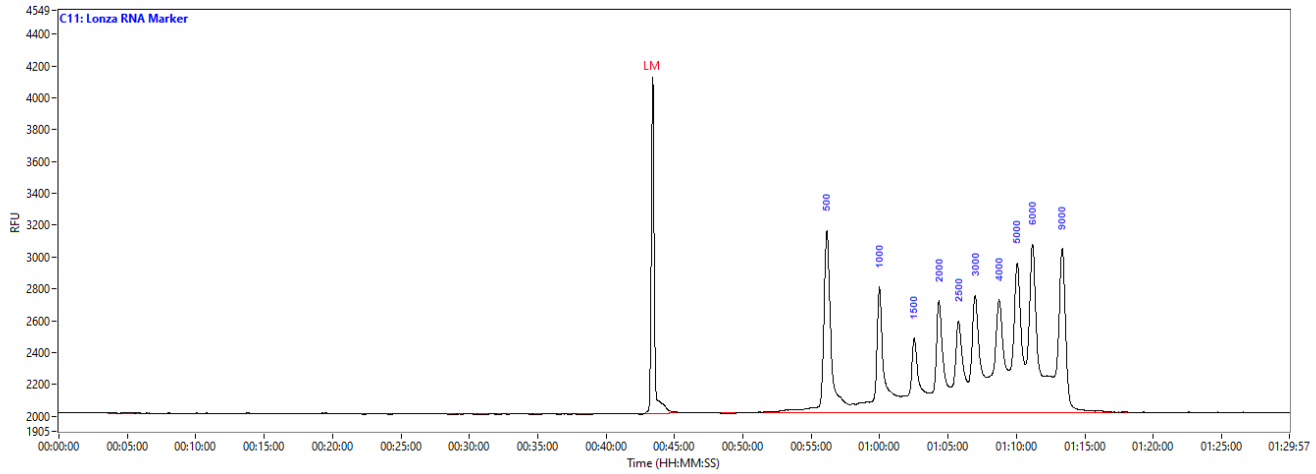
Typical result for a ~1,800 nt transcript at 5 ng/µL total input concentration. A Smear Analysis has been performed, as indicated by the dashed lines, between 1,600 and 2,800 nt. This allows the user to determine the percent purity and concentration of their peak of interest. The first peak corresponds to the 15 nt lower marker peak (LM).




Electropherogram of an ~1,800 nt IVT mRNA sample result using the Fragment Analyzer system with the DNF-472 RNA kit (15 nt) and the DNF-472B33 – HS IVT mRNA Mid Concentration run method. Peaks annotated by size (nt).

## IVT mRNA extended separation method with Lonza RNA Marker

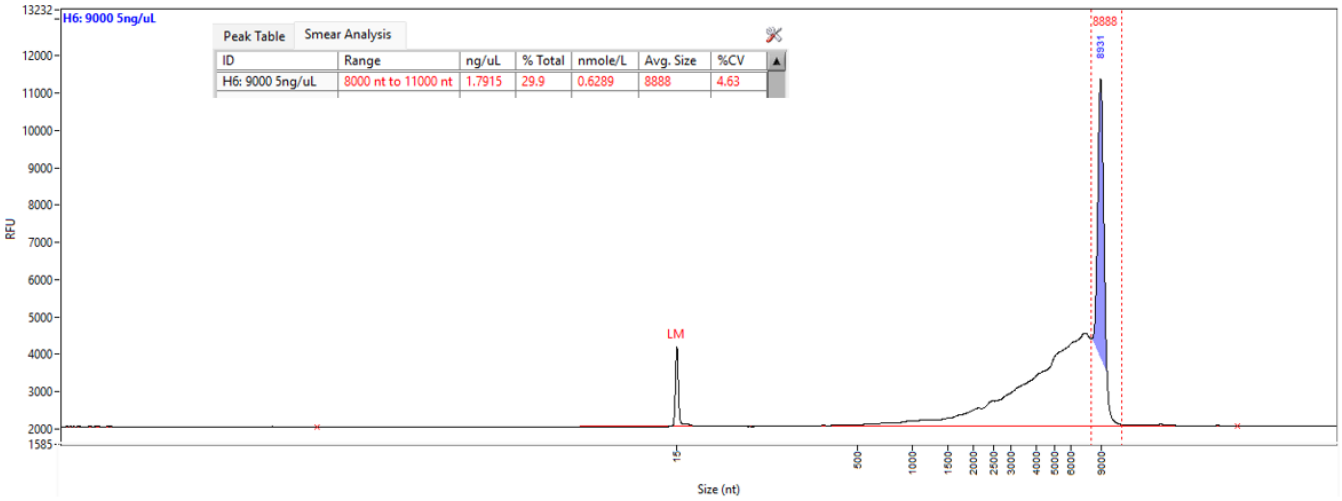
Typical result for the Lonza RNA Marker at the recommended dilution of 2 ng/µL. A total of 11 peaks should be observed with the sizes annotated in the figure below. The first peak corresponds to the 15 nt lower marker peak (LM).




Representative result for the Lonza RNA Marker diluted to 2 ng/µL using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt) and the DNF-472BE33 – HS IVT mRNA Extended Mid Concentration run method. Peaks annotated by size (nt).

## IVT mRNA sample: Smear analysis table using extended separation method

The figure below shows a typical result for a ~9,000 nt transcript at 5 ng/µL total concentration, using the Lonza RNA Marker. A Smear Analysis has been performed, as indicated by the dashed lines, between 8,000 and 11,000 nt. This allows the user to determine the percent purity and concentration of their peak of interest. The first peak corresponds to the 15 nt lower marker peak (LM).




Electropherogram of a ~9,000 nt IVT mRNA sample using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt) and the DNF-472BE33 – HS IVT mRNA Extended Mid Concentration run method. Peaks annotated by size (nt).

## Troubleshooting

The following table lists several potential kit 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.

| Issue | Cause | Corrective Action |
|---|---|---|
| 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 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 HS RNA kit (15 nt). 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 or a wrong rinse buffer is used. | 6 Prepare a new rinse buffer plate with 200 µL/well 0.25x TE buffer. |
| | 7 Array was contaminated. | 7 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for details). |
| Sample signal drops abruptly at the end of separation. | 1 Separation concentration too high and out of range. | 1 Verify sample was within concentration range specified for the HS RNA kit (15 nt). |
| Missing 6000 nt fragment in ladder. | 1 No rinse buffer in Marker plate; wrong rinse buffer. | 1 Use a fresh rinse buffer plate with 200 µL/well 0.25x TE buffer. |
| | 2 Dirty array inlet. | 2 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for details). |
| | 3 Aging array. | 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. |
| | 2 Sample was not heat denatured. | 2 Heat denature sample/ladder plate at 70°C for 2 minutes. |
| Peak too broad, signal too low and/or migration time too long. | 1 Capillary array needs to be reconditioned. | 1 Flush array with 0.5 N NaOH solution and repeat experiment. (See Appendix – Capillary Array Cleaning of the |

| | | |
|---|---|---|
| | 2 Capillary array vent valve is clogged. | Fragment Analyzer User Manual for details).<br>2 Clean vent valve with deionized water (See Fragment Analyzer User Manual for details). |
| No sample peak or marker peak observed for individual sample. | 1 Air trapped at the bottom of the sample plate well, or bubbles present in sample well.<br>2 Insufficient sample volume. A minimum of 20 µL is required.<br>3 Capillary is plugged. | 1 Check sample plate wells for trapped air bubbles. Centrifuge plate.<br>2 Verify proper volume of solution was added to sample well<br>3 Check waste plate for liquid in the capillary well using a 96-deepwell plate. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array. |

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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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