Agilent DNF-472 (15 nt) HS RNA Kit
- Total RNA and ribo-depleted RNA

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 is designed to analyze Total RNA samples at an input concentration of 50 pg/µL – 5,000 pg/µL and ribo-depleted RNA at an input sample concentration of 500 pg/µL – 5,000 pg/µL . A separate quick guide (D0117260) covers the use of the DNF-472 HS RNA kit for analyzing *in vitro* transcribed (IVT) mRNA.

Specifications

Analytical Specifications[1,]	HS RNA (15 nt) Kit	
Sizing Range	200 nt – 6,000 nt	
Sizing Accuracy[1]	± 20%	
Sizing Precision[1]	20% CV	
Limit of Detection (S/N > 3)	50 pg/µL – Total RNA	250 pg/µL – ribo-depleted RNA
Quantitative Range (per smear)	50 pg/µL – 5,000 pg/µL – Total RNA	500 pg/µL – 5,000 pg/µL – ribo-depleted RNA
Quantification Accuracy[1]	± 30%	
Quantification Precision[1]	20% CV	
Physical Specifications		
Total Electrophoresis Run Time	22cm[3]: 31 minutes, 33cm: 40 minutes, 55cm: 70 minutes	
Samples Per Run	12, 48 or 96; depending on the instrument type	
Sample Volume Required	2 µL	
Guaranteed Shelf Life	4 months	

[1] Results using RNA Ladder as sample.
[2] The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

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.

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.

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

Instrument	Compatible Arrays	Part Number
5200 Fragment Analyzer	FA 12 Capillary Array Ultrashort FA 12 Capillary Array Short FA 12 Capillary Array Long	A2300-1250-2247 A2300-1250-3355 A2300-1250-5580
5300 Fragment Analyzer.	FA 48 Capillary Array Short FA/ZAG 96 Capillary Array Short FA/ZAG 96 Capillary Array Long	A2300-4850-3355 A2300-9650-3355 A2300-9650-5580
5400 Fragment Analyzer	FA/ZAG 96 Capillary Array Short FA/ZAG 96 Capillary Array Long	A2300-9650-3355 A2300-9650-5580

Software

- Fragment Analyzer controller software
- ProSize data analysis software

Reagents

- Capillary Storage Solution (GP-440-0100)

Additional equipment required (not supplied)

- 96-well PCR sample 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 seal
- Sub-micron filtered DI water system: for 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 for sample dilutions
- RNaseZap (Ambion #AM9782 or equivalent product)
- Thermal cycler

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

Environmental conditions	• Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep instrument reagents at room temperature during sample preparation.
Sample Input Concentration	• Ensure sample input concentrations lie within kit specifications • Sample signal should not exceed 60,000 RFU.
Working with RNA Samples	• Ensure all working areas, reagents and plastic ware are RNase free. • Handle RNA samples with care. • Wear gloves at all times. • Thaw RNA samples on ice • Vortex and centrifuge all samples before use • Store RNA samples on ice throughout the preparation and analysis procedure
Steps before sample preparation	• Allow instrument reagents to equilibrate at room temperature for 30 min prior to use. • Thaw HS RNA Ladder on ice prior to use. • Keep HS RNA Diluent Marker on ice and in the dark before 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. • 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. • Thaw a 3 µL 25 ng/µL ladder aliquot on ice. • Spin down the contents and mix by pipetting the solution up and down with a pipette tip set to a 2 µL volume. 1. Transfer 2 µL of the 25 ng/µL Ladder to a fresh Eppendorf LoBind 0.5 mL tube. 2. Heat denature the ladder at 70°C for 2 minutes, immediately cool to 4°C and keep on ice. • 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 working 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 • 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. • The working concentration of 2 ng/µL HS RNA Ladder must be run in parallel with the samples for each experiment to ensure accurate quantification.
Total RNA Sample Preparation	• Heat-denature the RNA samples at 70°C for 2 minutes if needed and immediately cool to 4°C and keep on ice before use. • The total RNA input sample must be within a total concentration range of **50 pg/µL – 5,000 pg/µL** for optimal kit results. If the concentration of the sample is above this range, dilute with RNase-free water.
Ribo-depleted RNA Sample Preparation	• Heat-denature the ribo-depleted RNA samples at 70°C for 2 minutes if needed and immediately cool to 4°C and keep on ice before use. • The ribo-depleted RNA sample must be within a total concentration range of **250 pg/µL – 5,000 pg/µL** for 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 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 of blank solution. • Pipette 2 µL of each heat-denatured RNA sample into the respective wells of the sample plate. • Thaw the denatured 2 ng/µL working concentration of the RNA Ladder on ice. • Pipette 2 µL of working concentration denatured RNA Ladder into the 18 µL of DM solution in the designated ladder well.

Mixing and centrifugation recommendations	• When mixing sample with Diluent Marker (DM), mix the contents of the well thoroughly**.** It is suggested to perform ***one*** of the following methods to ensure complete mixing: · 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. · 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. · Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample or ladder volume. • After mixing, centrifuge the plate to remove any air bubbles. • 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 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	15
48	25
96	45
192	80
384	160

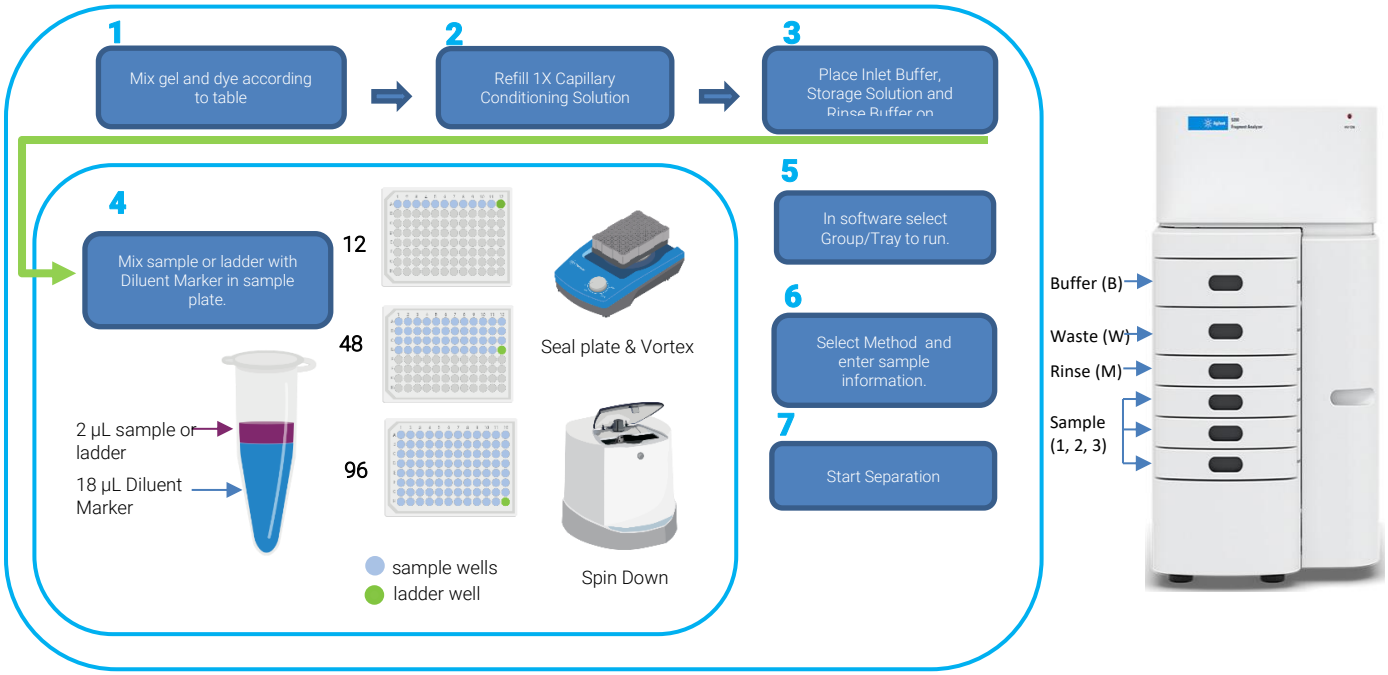
Agilent HS RNA DNF-472 Kit Operating Procedure

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 a 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

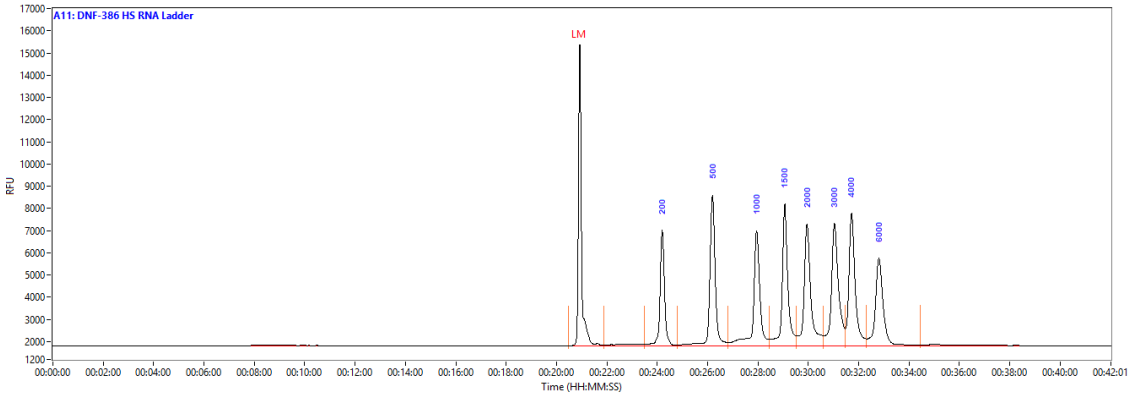
   Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.
   - 5200 – row H, 1 mL/well, drawer B
   - 5300 – 48 capillary, rows A-D, 100 µL/well, drawer 3
   - 5300/5400 – 96 capillary, all rows, 100 µL/well, drawer 3

   Place 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 or 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.
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;
   - DNF-472T22 (for total RNA analysis on Capillary Array Ultrashort)
   - DNF-472M22 (for ribo-depleted RNA analysis on Capillary Array Ultrashort)
   - DNF-472T33 (for total RNA analysis on Capillary Array Short)
   - DNF-472M33 (for ribo-depleted RNA analysis on Capillary Array Short)
   - DNF-472T55 (for total RNA analysis on Capillary Array Long)
   - DNF-472M55 (for ribo-depleted RNA analysis on Capillary Array Long)

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

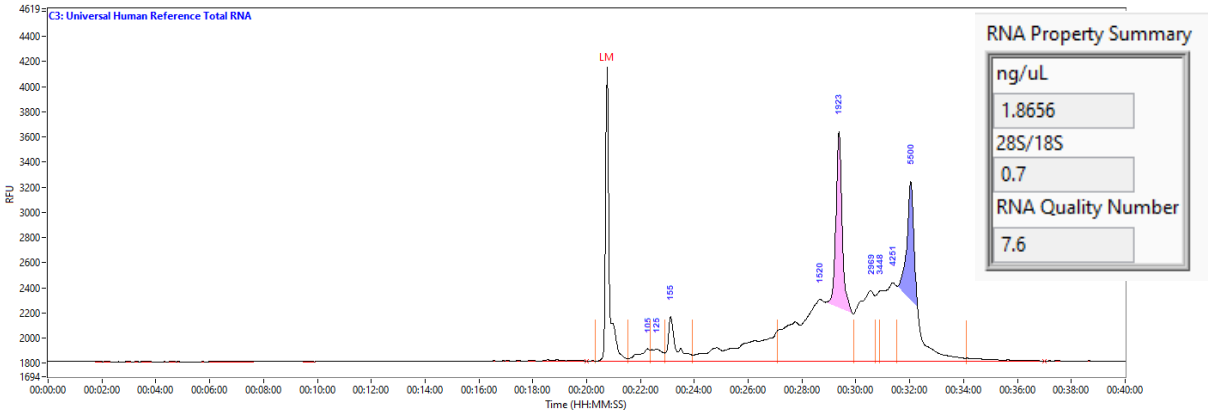


HS RNA Ladder Result



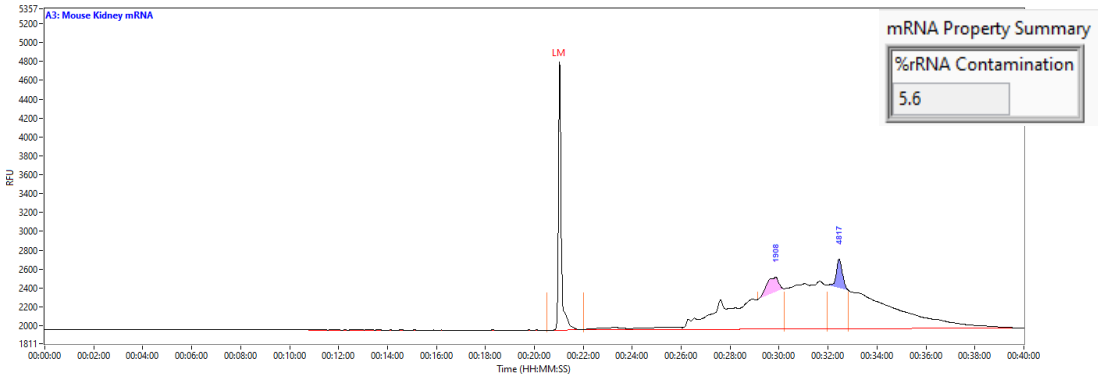
Representative HS RNA Ladder result using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt). Method: DNF-472T33 (Total RNA analysis method, Capillary Array Short). Peaks annotated by size (nt). RFU values may differ between instruments.

Total RNA Sample Result



Example Universal Human Reference total RNA sample result using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt). Method: DNF-472T33 (Total RNA analysis method, Capillary Array Short). Peaks annotated by size (nt). The **RNA Property Summary** is displayed for each sample in the Total RNA analysis mode in the ProSize data analysis software, reporting the total RNA concentration, 28S/18S ratio, and RNA Quality Number (RQN). RFU values may differ between instruments.

Ribo-Depleted RNA Sample Result



Example mouse kidney ribo-depleted RNA sample result using the Fragment Analyzer system with the DNF-472 HS RNA kit (15 nt). Method: DNF-472M33 (ribo-depleted mRNA analysis method, Capillary Array Short). Peaks annotated by size (nt). The **mRNA Property Summary** is displayed for each sample in the ProSize data analysis software, reporting %rRNA Contamination (% of ribosomal RNA in the total concentration). RFU values may differ between instruments.

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 Verify sample was correctly added and mixed to sample well.
	5 Sample not added to diluent marker solution or not mixed well.	6 Prepare a new rinse buffer plate with 200 µL/well 0.25x TE buffer.
	6 Rinse buffer is not fresh or a wrong rinse buffer is used.	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).
	7 Array was contaminated.	
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 25S or 28S ribosomal peak; 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.
	4 If sample is insect total RNA,heat denaturing can cause the 28S peak of some samples to cleave, causing incorrect RQN.	4 Run insect total RNA sample without heat denaturing.
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 RNA Sample or RNA Ladder not heat denatured.	2 Heat denature RNA samples and RNA Ladder at 70°C for 2 minutes and immediately cool on ice prior to analysis (refer to Essential Practices section).

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 Fragment Analyzer User Manual for details).
	2 Capillary array vent valve is clogged.	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.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate.
	2 Insufficient sample volume. A minimum of 20 µL is required.	2 Verify proper volume of solution was added to sample well
	3 Capillary is plugged.	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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Technical Support and Further Information
For technical support please visit www.agilent.com which offers useful information and support regarding the products and technology.

www.agilent.com

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