Agilent Technologies

Troubleshooting problems – Protein analysis

2100 Bioanalyzer

A08024.pdf

Troubleshooting the Protein Application

Essential Measurement Practices

For hints on how to handle chips and chemicals, see Essential Measurement Practices—7.

Troubleshooting the Protein Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see Symptoms—185.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause
- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

If you are not able to assign a symptom to your problem, compare your electropherogram with the List of Protein Electropherograms—231.

Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Clogged spin filters

Most Probable Causes	Solution
Gel was centrifuged at too low g-value.	Refer to the Protein Reagent Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for preparation of gel-dye mix and/or destaining solution.	Repeat centrifugation step without cooling.
Least Probable Causes	**Solution**
Particles in the gel-dye mix and/or destaining solution.	Repeat the preparation of the gel-dye mix and/or destaining solution. Wear powder-free gloves only.

Back to **Symptoms**

Too High Quantitation Results

Most Probable Causes	Solution
Upper marker wrongly assigned.	Check assignment of upper marker.
Diluted samples are too old.	Use diluted samples within one day.
Sample buffer and/or Denaturating Solution not handled according to the instructions.	For proper preparation and storage of the sample buffer and denaturating solution, refer to the Protein Ragent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	**Solution**
Samples not completly denaturated.	Use fresh sample aliquot. Heat sample/ denaturating solution for 5 min at 100°C.
Sample/denaturating solution are dried out.	Sample/denaturating solution were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.

Least Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 10 minutes.

Back to **Symptoms**

Too Low Quantitation Results

Most Probable Causes	Solution
Upper marker wrongly assigned.	Check assignment of upper marker.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	**Solution**
Sample concentration too high.	Use sample concentration according to the Protein Reagent Kit Guide. Don´t forget to dilute samples with deionized water after heat denaturation.
Diluted sample are too old.	Use diluted samples within one day.
Lease Probable Causes	**Solution**
Loaded chip kept too long before run.	Prepared chips must be used within 10 min.

Back to **Symptoms**

Wrong Sizing Result

Most Probable Causes	Solution
Ladder degraded.	Refer to the Reagent Kit guide for proper ladder storage. Optional: prepare ladder aliquot. Use a fresh ladder aliquot.
Upper and/or lower marker wrongly assigned.	Store Sample Buffer/Denaturating Solution according to the instructions given in the Reagent Kit Guide. See **Low or Missing Upper Marker in Ladder—200**
Ladder peaks wrongly assigned.	Check assignment of ladder peaks. See **Wrong Alignment of Ladder Peaks—222** for details.
Protein ladder not properly denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C.
Probable Causes	**Solution**
Protein ladder not properly denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C.

Least Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the **Hardware Diagnostics—48**. If the power supply is defective, call Agilent Technologies.

Back to **Symptoms**

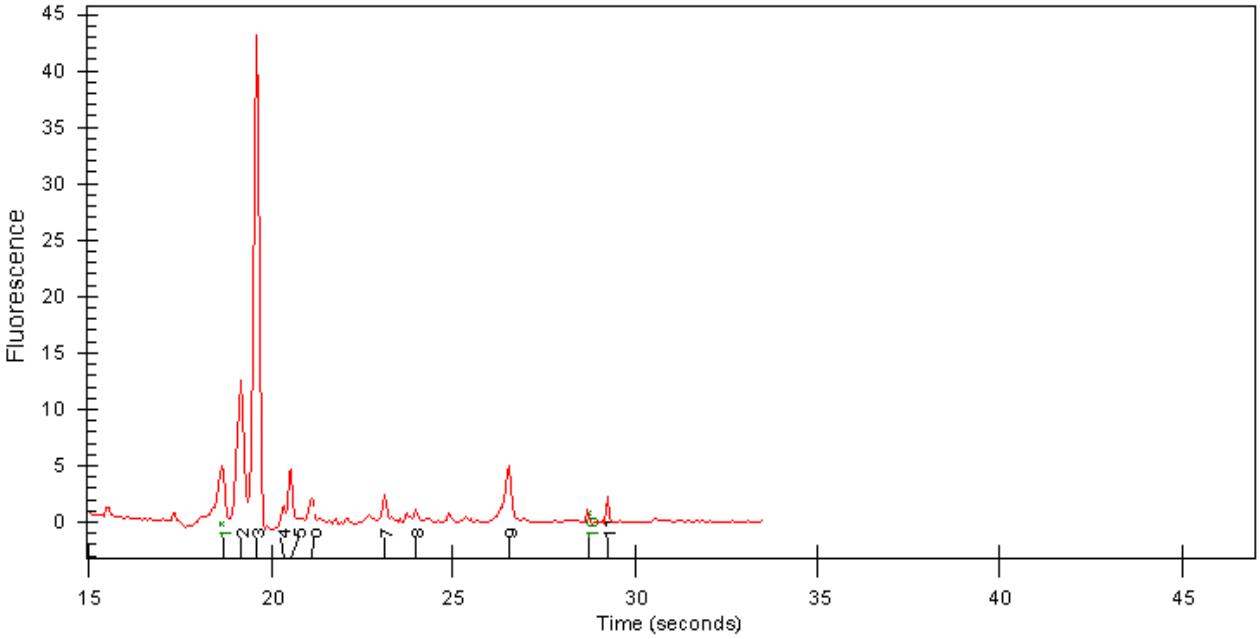
Poor Chip Performance

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see **Maintaining the Chip Priming Station—271**). Check if clip and base plate of priming station are in the right position (see Protein Reagent Kit Guide).
Amount of liquid pipetted is too low or chip is empty.	Check Reagent Kit Guide on amount of liquid to be pipetted. Fill unused wells with ladder or sample replicate. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.

Probable Causes	Solution
Chip preparation was done with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature before use.
Chips were stored in the fridge/freezer.	Prepare a new chip. Store chips at room temperature.
Least Probable Causes	**Solution**
High voltage power supply defective.	Check high voltage power supply using the **Hardware Diagnostics—48**. If the power supply is defective, call Agilent Technologies.

Back to **Symptoms**

Apparently Short Run Time



Show me how to solve **Apparently Short Run Time**

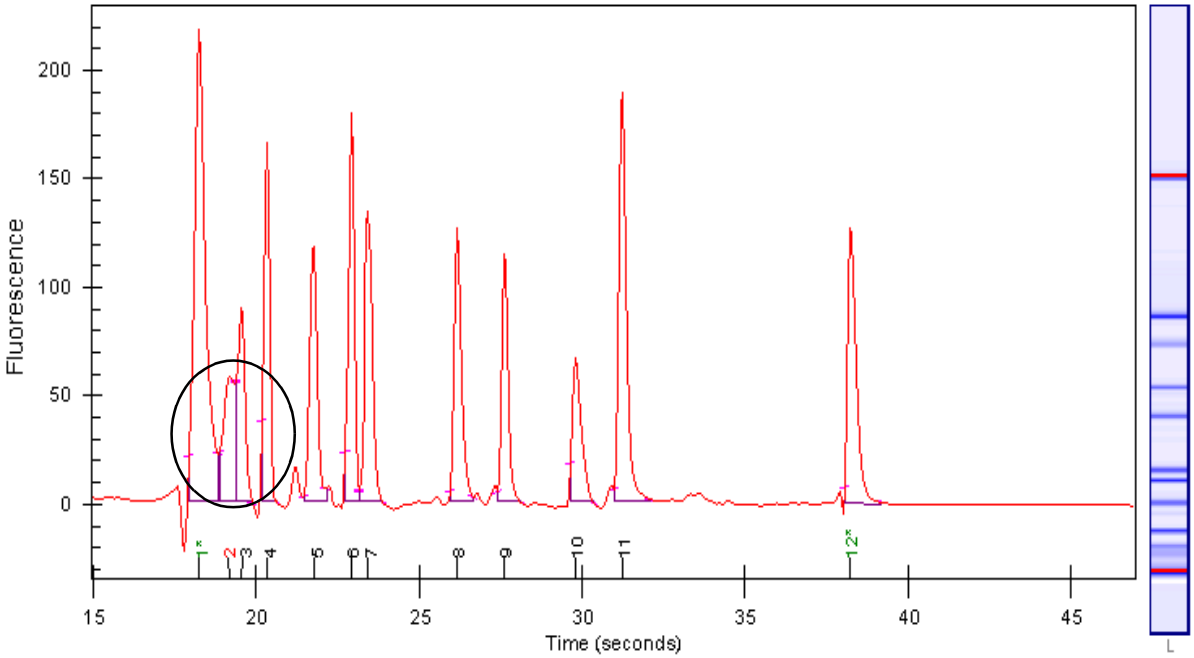
Back to **Symptoms**

Apparently Short Run Time

Most Probable Causes	Solution
Low intensity of upper marker in the ladder. They were not assigned correctly by the software.	To correct for wrong selected upper marker in ladder, set upper marker manually. If necessary, adjust peak find settings. If peaks are detected that are not part of the ladder, exclude them. For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker. See **Low or Missing Upper Marker in Ladder—201** for probable causes.

Back to **Symptoms**

Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks**

Back to **Symptoms**

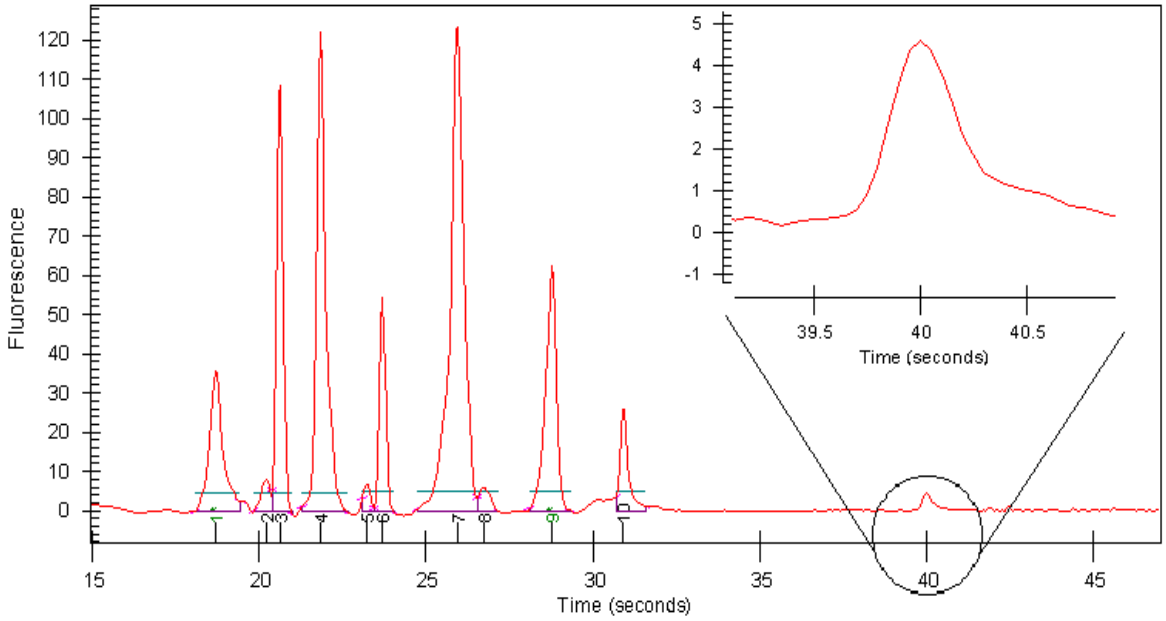
Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Sample or ladder not denaturated properly.	Use fresh sample aliquot. Heat sample/ denaturating solution and ladder for 5 min at 100°C
Sample/denaturating solution and/or ladder are dried out during denaturation.	Sample/denaturating solution and/or ladder were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturating
Chip contaminated. Dust particles in separation channels.	Wear powder-free gloves only. Don't touch the wells of the chip. Clean the electrodes. See Maintenance—246 for additional information. Load the chip immediately after taking it out of its sealed bag.

Probable Causes	Solution
Ladder degraded.	Refer to the Protein Reagent Kit Guide for proper ladder storage. Optional: Prepare ladder aliquots. Use a new aliquot.
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.

Back to **Symptoms**

Low or Missing Upper Marker in Ladder



Show me how to solve **Low or Missing Upper Marker in Ladder**

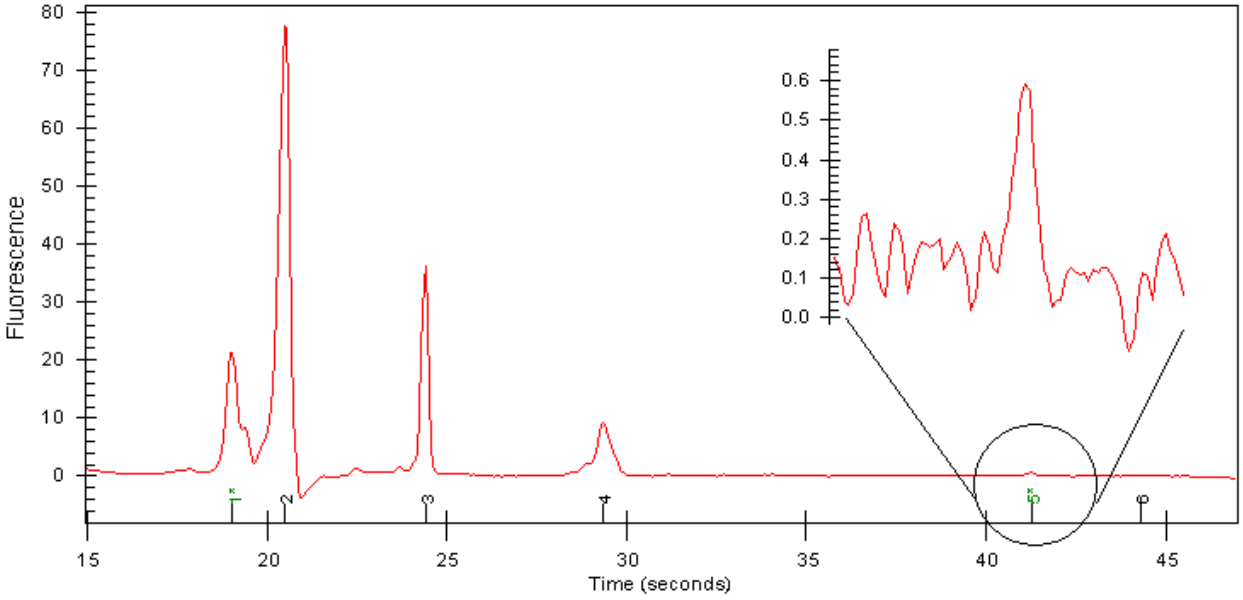
Back to **Symptoms**

Low or Missing Upper Marker in Ladder

Most Probable Causes	Solution
Ladder degraded.	For correct ladder storage and denaturation, refer to the Protein Reagent Kit Guide. To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings. If peaks are detected that are not part of the ladder, exclude them. For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.
Diluted ladder is too old.	Use diluted ladder within one day.
Probable Causes	**Solution**
Ladder not denaturated properly.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C
Ladder dried out during denaturation.	Ladder was denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.

Back to **Symptoms**

Low or Missing Upper Marker in Sample



Show me how to solve **Low or Missing Upper Marker in Sample**

Back to **Symptoms**

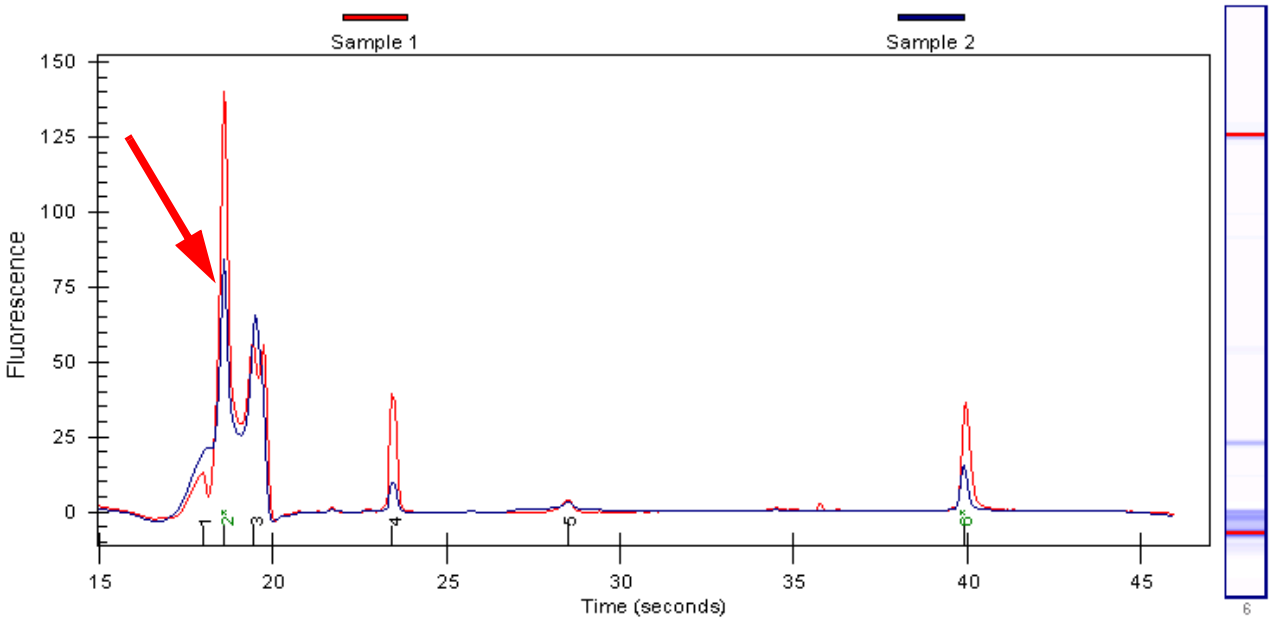
Low or Missing Upper Marker in Sample

Most Probable Causes	Solution
Sample buffer/denaturating solution not handled according to the instructions.	Refer to the instructions provided with the Reagent Kit guide for storage and preparation of the sample buffer/denaturating solution. To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings. For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.
Incompatible sample component. Some components of the buffer, e.g. CHAPS, TFA, etc. interfere with the upper marker and decrease sensitivity.	See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site www.agilent.com/chem/labonachip. If necessary dilute, dialyze or desalt the sample. It is recommended to dilute the samples 1:2, 1:4, ... with water to find the optimal dilution.
Diluted samples are too old.	Use diluted samples within one day.

Probable Causes	Solution
Samples not denaturated properly.	Use fresh sample aliquot. Heat samples with denaturating solution for 5 min at 100°C
Samples dried out during denaturation.	Samples were denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.
Least Probable Causes	**Solution**
Upper marker was digested by proteases (cell lysates).	Add protease inhibitor cocktails to cell lysate samples.

Back to **Symptoms**

High Lower Marker Variability



Show me how to solve **High Lower Marker Variability**

Back to **Symptoms**

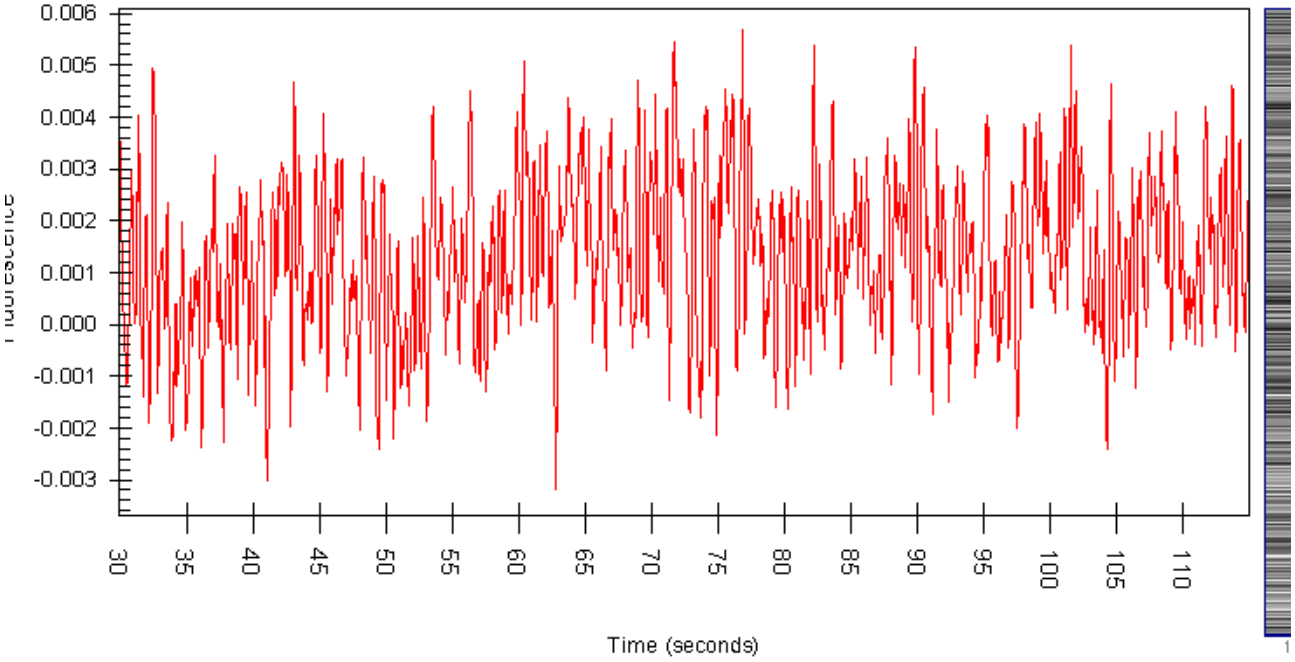
NOTE As long as the lower marker is detected, the assay performance is not affected by lower marker variability.

High Lower Marker Variability

Most Probable Causes	Solution
Buffer components of the sample , e.g. salts, detergents, other additives etc. interfere with the lower marker. Variability of ionic strength of the sample influence the lower marker intensity.	See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds. If necessary dilute, dialyze or desalt the sample.

Back to **Symptoms**

No Peaks



Show me how to solve **No Peaks**

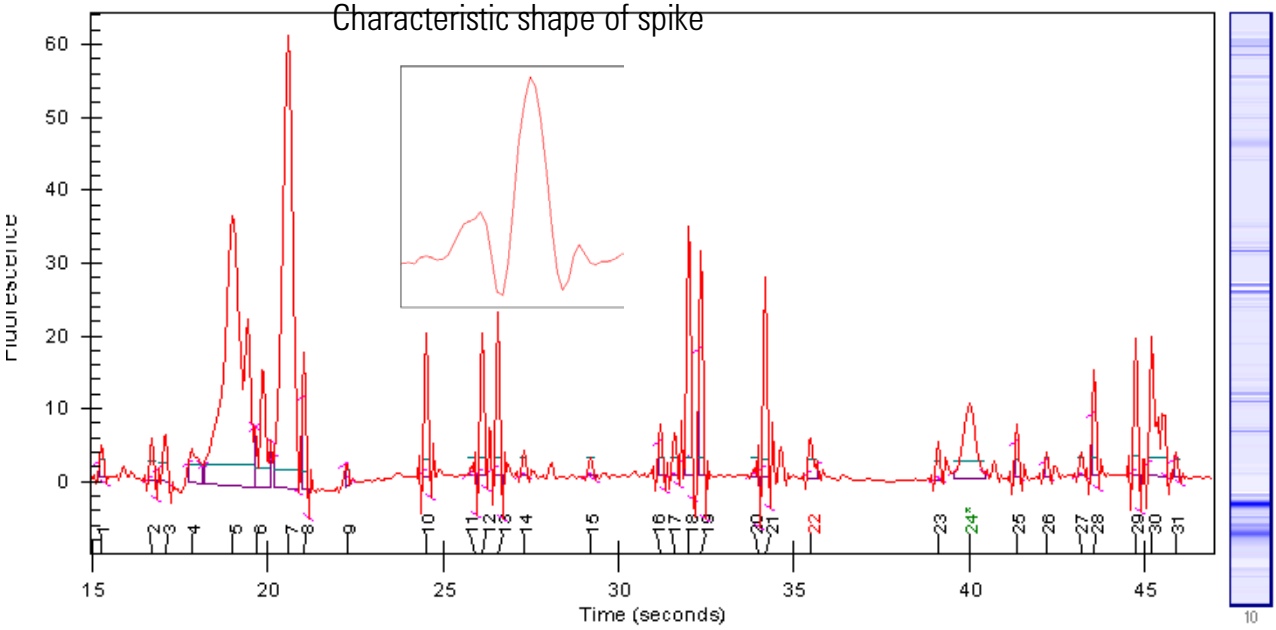
Back to **Symptoms**

No Peaks

Most Probable Causes	Solution
Laser defective.	Check laser using the **Hardware Diagnostics—48**. If the laser is defective, call Agilent Technologies.
Gel dye mix was loaded in the destain well instead of destaining solution.	Discard chip and prepare new chip according to protocol.
Probable Causes	**Solution**
Autofocus failure.	Check autofocus using the **Hardware Diagnostics—48**. If autofocus fails, call Agilent Technologies.
Least Probable Causes	**Solution**
High voltage power supply defective.	Check high voltage power supply using the **Hardware Diagnostics—48**. If the power supply is defective, call Agilent Technologies.
Fingerprint on focusing lens.	Clean lens like decribed in **Lens Maintenance—270**.

Back to **Symptoms**

Spikes



Show me how to solve Spikes

Back to **Symptoms**

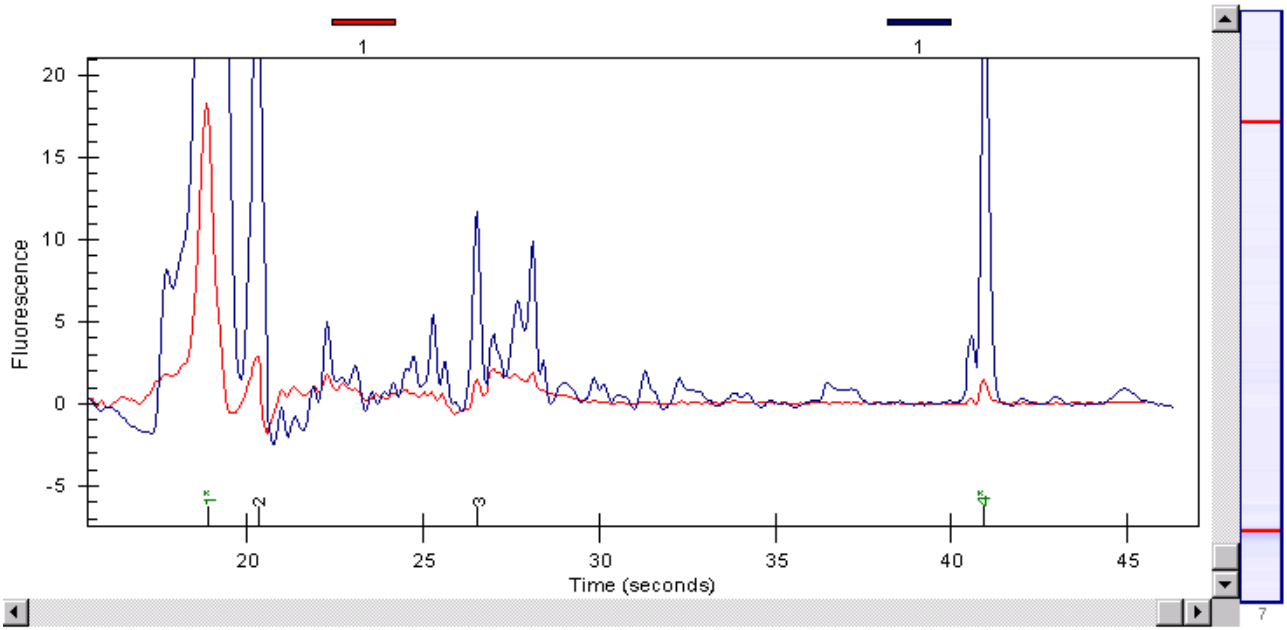
Spikes

Most Probable Causes	Solution
Chip/gel-dye mix/destaining solution contaminated.	Prepare new chip with new gel-dye mix and new destaining solution: Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Gel-dye mix/destaining solution not properly prepared.	Refer to the Reagent Kit Guide for proper preparation of the gel-dye mix and destaining solution. Let the dye warm up to room temperature for 20 min before preparing the gel-dye mix.
Chip not properly prepared.	Prepare a new chip. Allow all reagents and samples to warm up to room temperature before use.

Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Least Probable Causes	**Solution**
Power outlett	Install power filter.

Back to **Symptoms**

Poor Reproducibility



Show me how to solve Poor Reproducibility

Back to **Symptoms**

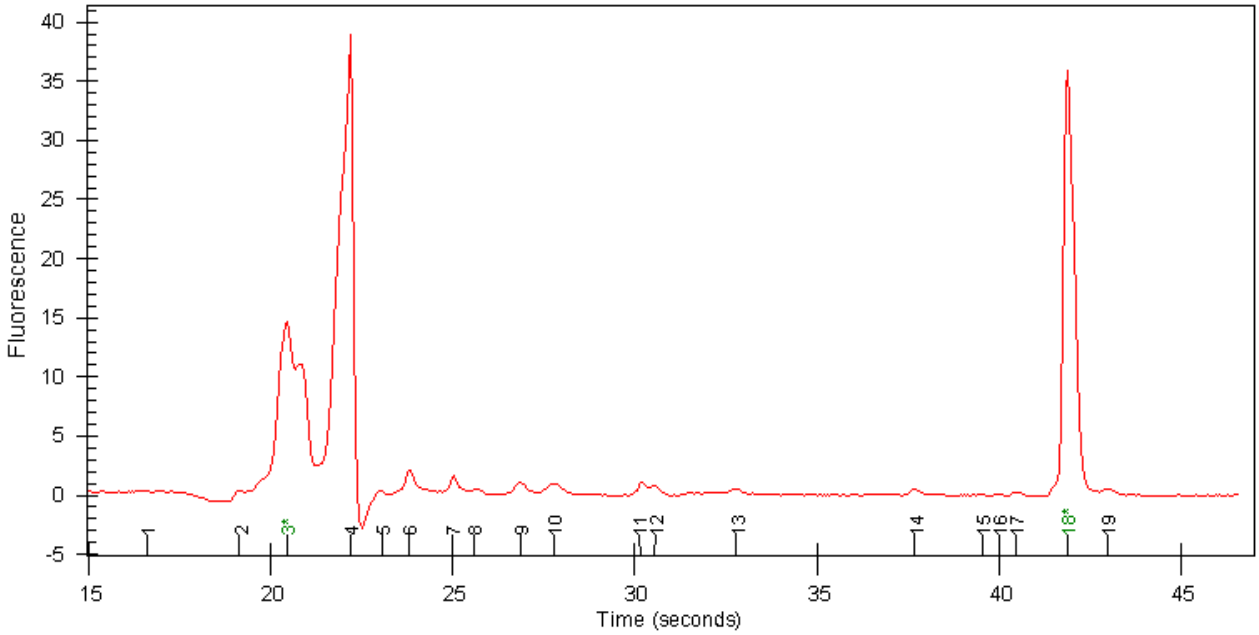
Poor Reproducibility

Most Probable Causes	Solution
Wrong peak alignment.	Check if alignment is correct (wrong alignment might cause broad peaks compared to the rest of the chip). For better identification of the lower and upper marker: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the lower and upper marker.
One Sample not denaturated properly.	Use fresh sample aliquot. Heat samples with denaturating solution for 5 min at 100°C
One Sample dried out during denaturation.	Samples were denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.
Reducing agent (BME or DTT) was added in one sample and not in the other.	Refer to the Reagent Kit Guide for proper sample reduction.

Probable Causes	Solution
Diluted samples are too old.	Use diluted samples within one day.
Buffer component interfers with LDS/SDS in sample buffer.	See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site www.agilent.com/chem/labonachip. If necessary dilute, dialyze or desalt the sample.

Back to **Symptoms**

No or Low Sample Peaks



Show me how to solve **No or Low Sample Peaks**

Back to **Symptoms**

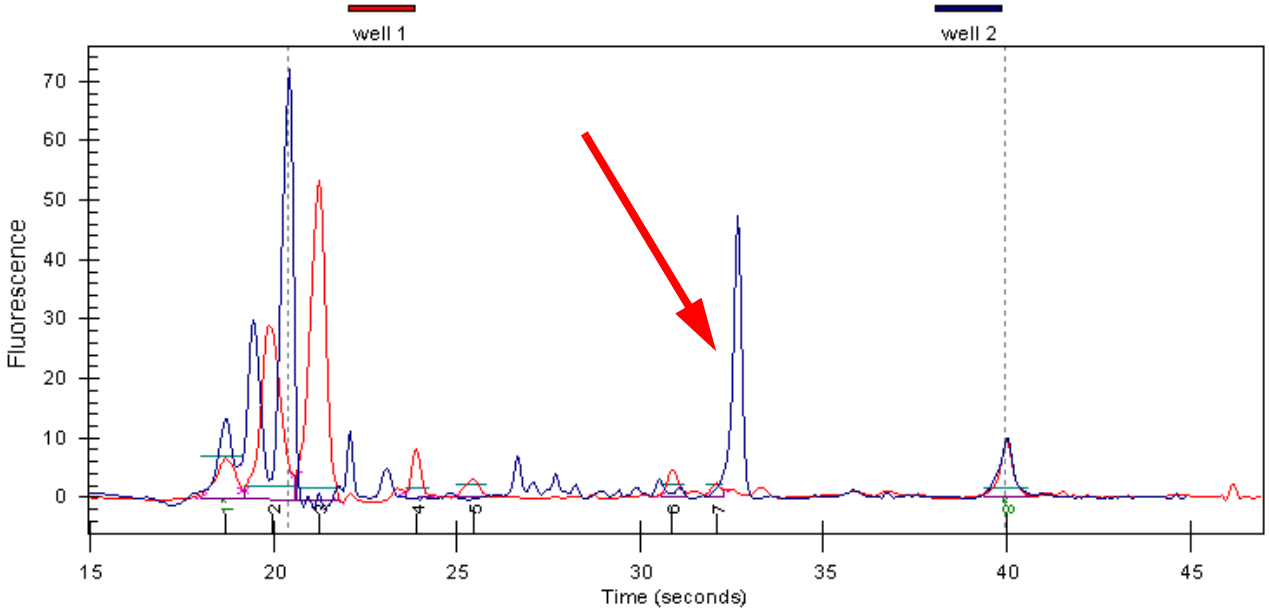
No or Low Sample Peaks

Most Probable Causes	Solution
Protein concentration in samples too low.	Use protein concentration accorting to specifications given in the Reagent Kit Guide.
Too high salt concentration in samples.	Sensitivity is strongly affected by salt concentration. Dilute samples in deionized H_2O, dialyze samples against low salt buffer or desalt samples using spin filters.
SDS not completely dissolved in dye concentrate.	Let dye concentrate equilibrate to room temperature for 20 min before use. Check for undissolved SDS cristals in the tube. Vortex dye concentrate well before use. If necessary heat the sample buffer to 37°C for 2 min.
Probable Causes	**Solution**
Samples not completly denaturated.	Use fresh sample aliquot. Heat sample/ denaturating solution for 5 min at 100°C.
Sample/denaturating solution are dried out.	Sample/denaturating solution were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Diluted samples are too old.	Use diluted samples within one day.

The gel dye mix was not replaced after priming the chip.	Prepare new chip according to the Protein 200 Reagent Kit Guide.
Least Probable Causes	**Solution**
Samples dissolved in acidic buffer.	Neutralize samples with appropriate buffer or dilute samples in deionized H_2O. Alternatively dialyze samples against buffer with medium pH.
Autofocus failure.	Check autofocus by means of the **Hardware Diagnostics—48**. If autofocus fails, call Agilent Technologies.

Back to **Symptoms**

Apparently Missing Sample Peak



Show me how to solve **Apparently Missing Sample Peak**

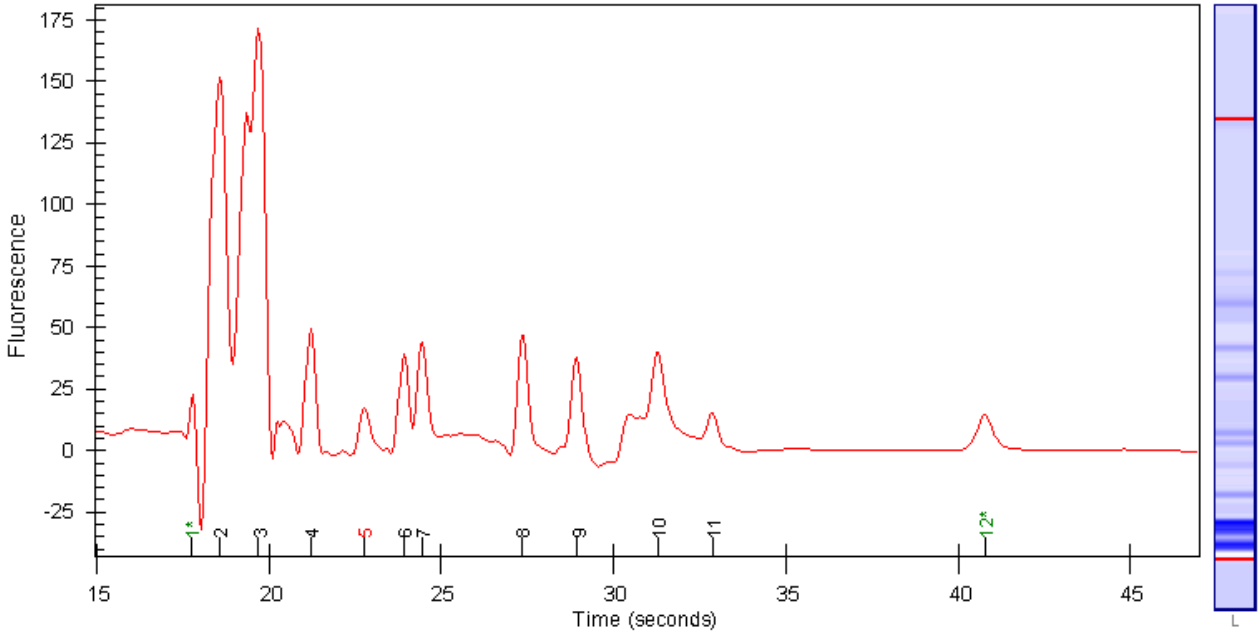
Back to **Symptoms**

Apparently Missing Sample Peak

Most Probable Causes	Solution
Wrongly assigned upper marker. Sample buffer/denaturating solution not handled according to the instructions. Because of low intensity, the software identified sample peak as upper marker.	Refer to the instructions provided with the Reagent Kit guide for storage and preparation of the sample buffer/denaturating solution. To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings. For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.

Back to **Symptoms**

Low Ladder Peaks



Show me how to solve **Low Ladder Peaks**

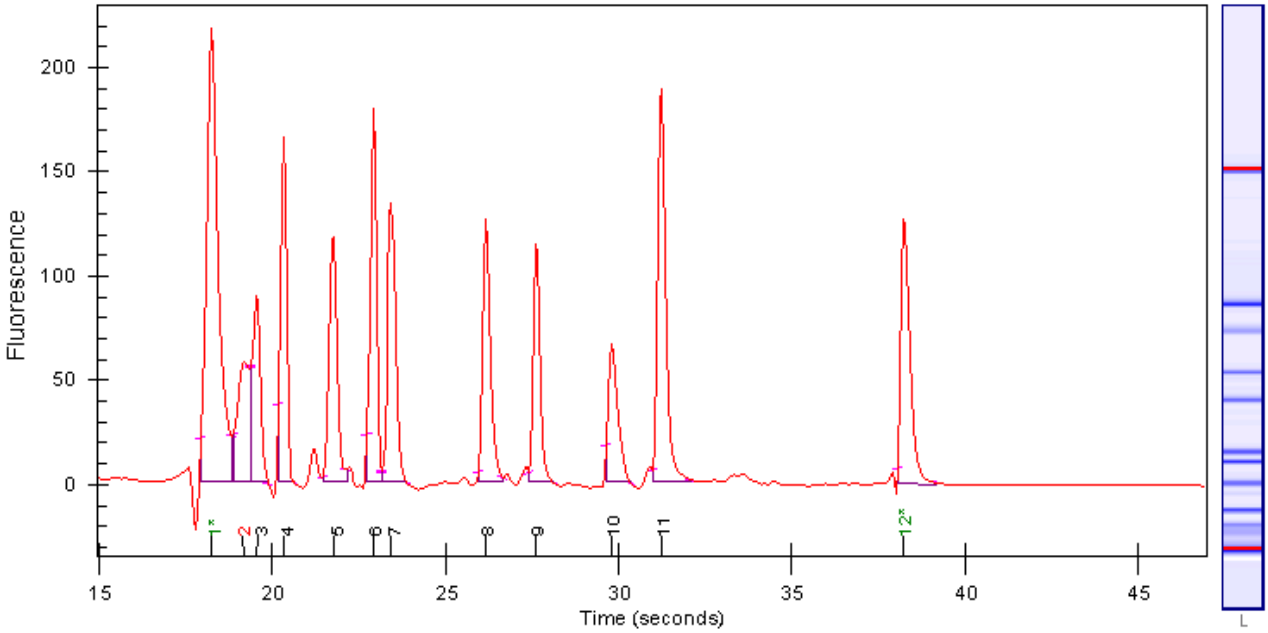
Back to **Symptoms**

Low Ladder Peaks

Most Probable Causes	Solution
Ladder degraded.	Refer to the Protein Reagent Kit Guide for proper ladder storage. Optional: Prepare ladder aliquots. Use a new aliquot.
Ladder not diluted after denaturation.	Refer to the Ragent Kit Guide for proper chip preparation.
Probable Causes	**Solution**
Ladder not completly denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C.
Ladder dried out.	Ladder was denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.
Diluted ladder is too old.	Use diluted ladder within one day.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.

Back to **Symptoms**

Wrong Alignment of Ladder Peaks



Show me how to solve **Wrong Alignment of Ladder Peaks**

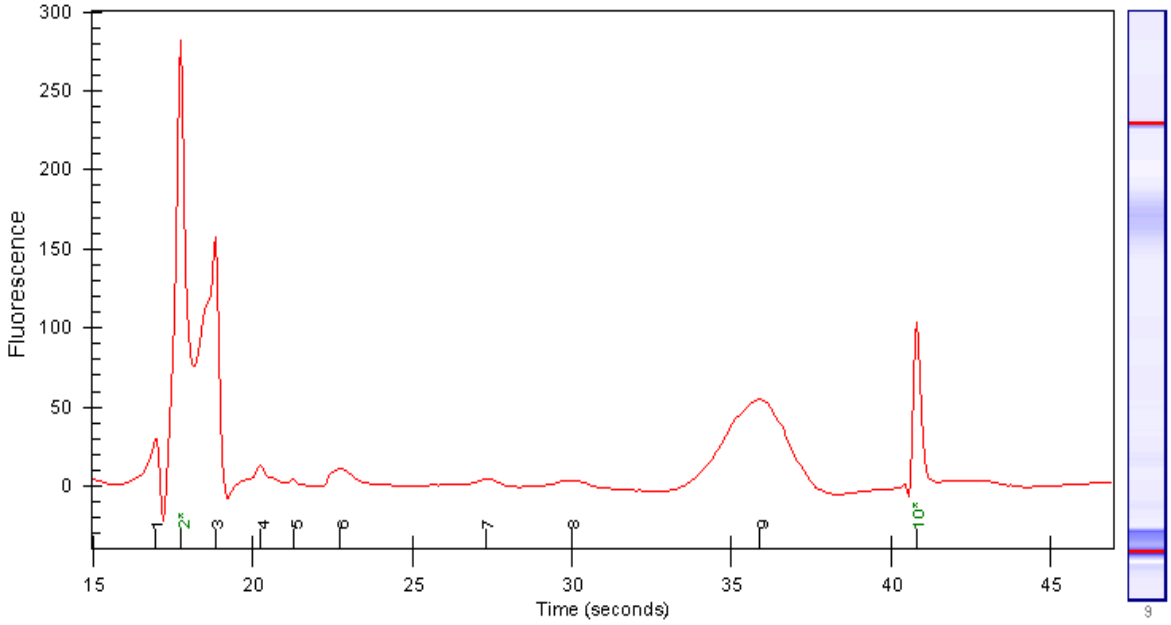
Back to **Symptoms**

Wrong Alignment of Ladder Peaks

Most Probable Causes	Solution
Low intensity of upper marker. The software identifies ladder peak as upper marker.	If necessary adjust peak find settings and exclude low intensity peaks. See Low Ladder Peaks—221 for probable causes. For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.

Back to Symptoms

Broad Peaks



Show me how to solve **Broad Peaks**

Back to **Symptoms**

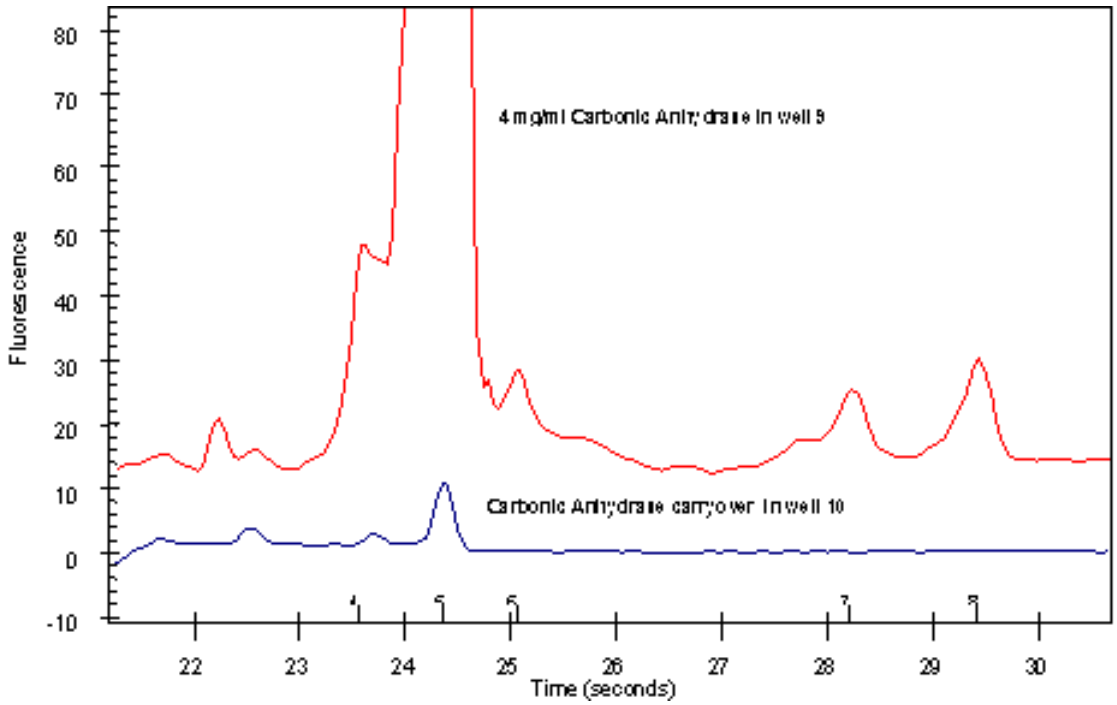
Broad Peaks

Most Probable Causes	Solution
Wrong peak alignment.	Check if alignment is correct (wrong alignment might cause broad peaks compared to the rest of the chip). For better identification of the lower and upper marker: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the lower and upper marker.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see **Maintaining the Chip Priming Station—271**). Check if clip and base plate of priming station are in the right position (see Protein Reagent Kit Guide).
Leak Current due to contaminated electrodes. Chip was left in instrument after run.	Clean electrodes with analysis-grade water and a toothbrush, see **Maintenance—246**. Don´t leave chip in instrument after run. Clean electrodes after each run. Replace electrode cartridge.

Probable Causes	Solution
Sample was not denaturated properly.	Use fresh sample aliquot. Heat sample/ denaturating solution for 5 min at 100°C.
Reducing agent (BME or DTT) was added in one sample and not in the other.	Refer to the Reagent Kit Guide for proper sample reduction.
High voltage power supply defective.	Check high voltage power supply using the **Hardware Diagnostics—48**. If the power supply is defective, call Agilent Technologies.

Back to **Symptoms**

Cross Contamination



Show me how to solve **Cross Contamination**

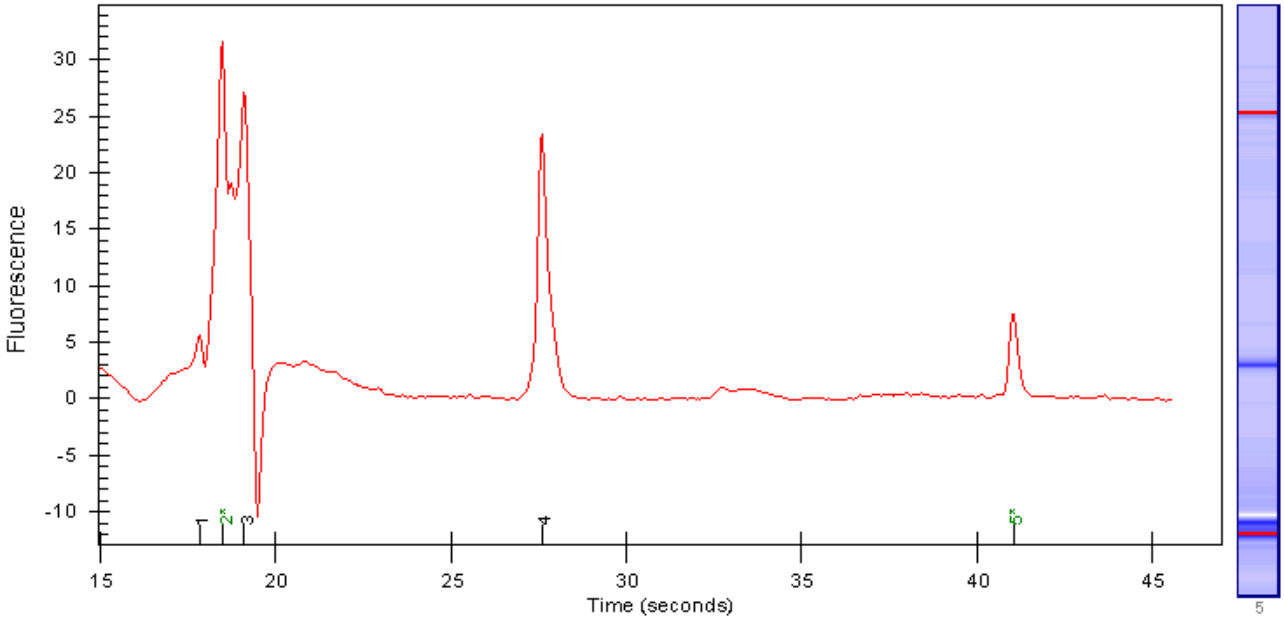
Back to **Symptoms**

Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the Protein Reagent Kit Guide.
Contaminated electrodes. Chip left in instrument after run.	Clean electrodes with analysis-grade water and a toothbrush, see **Maintenance—246**. Dont´t leave chip in instrument after run. Clean electrodes after each run.
Probable Causes	**Solution**
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip and pipette again. Use appropriate pipette and tips.

Back to **Symptoms**

Dips



NOTE As long as the lower marker is detected, the assay performance is not affected by dips.

Show me how to solve **Dips**

Back to **Symptoms**

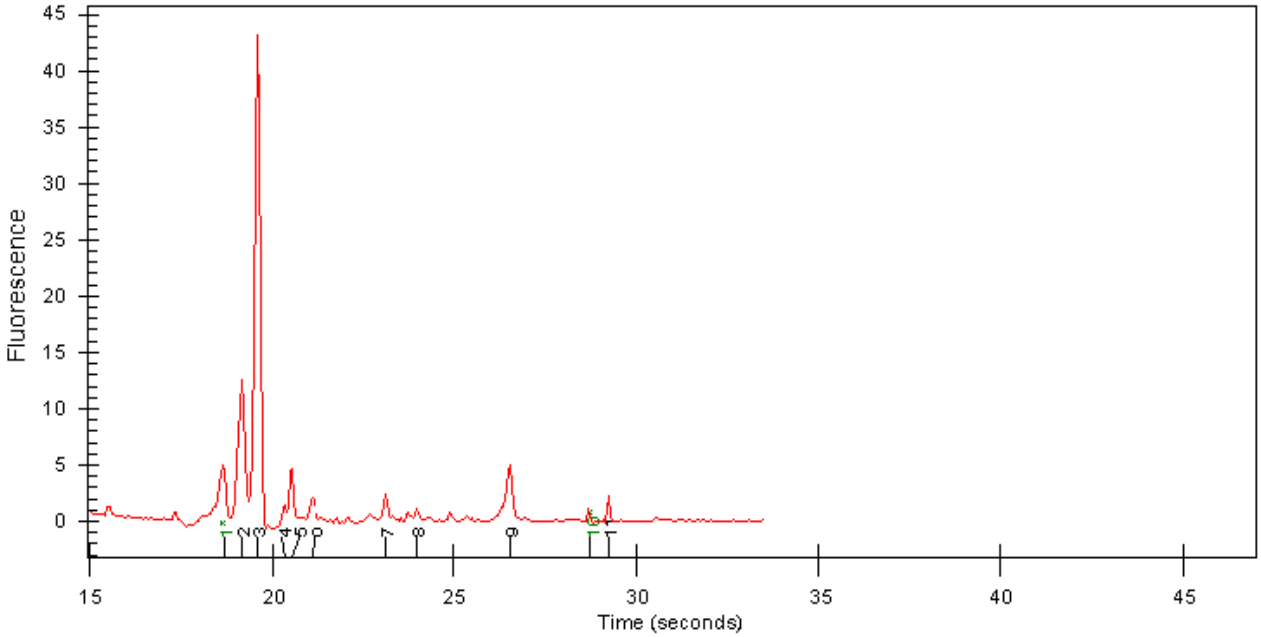
Dips

Most Probable Causes	Solution
Sample contains additional detergents and/or dyes.	See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site www.agilent.com/chem/labonachip. If necessary dilute, dialyze or desalt the sample.

Back to **Symptoms**

List of Protein Electropherograms

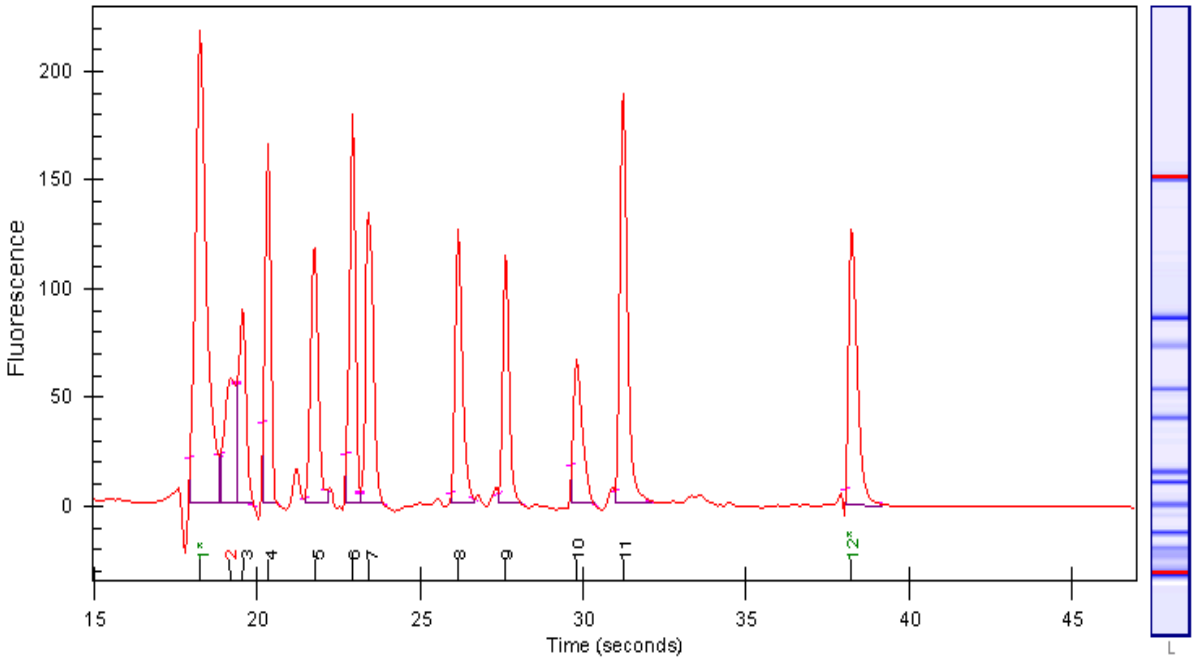
Apparently Short Run Time



Show me how to solve **Apparently Short Run Time**

Back to the **Top of List**

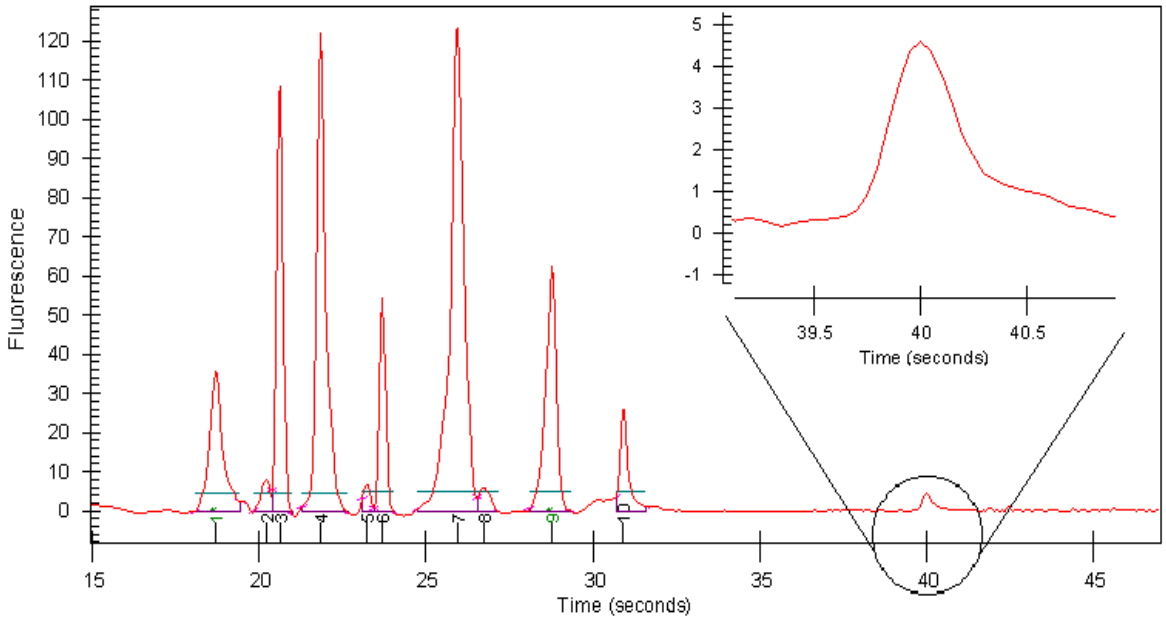
Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks**

Back to the **Top of List**

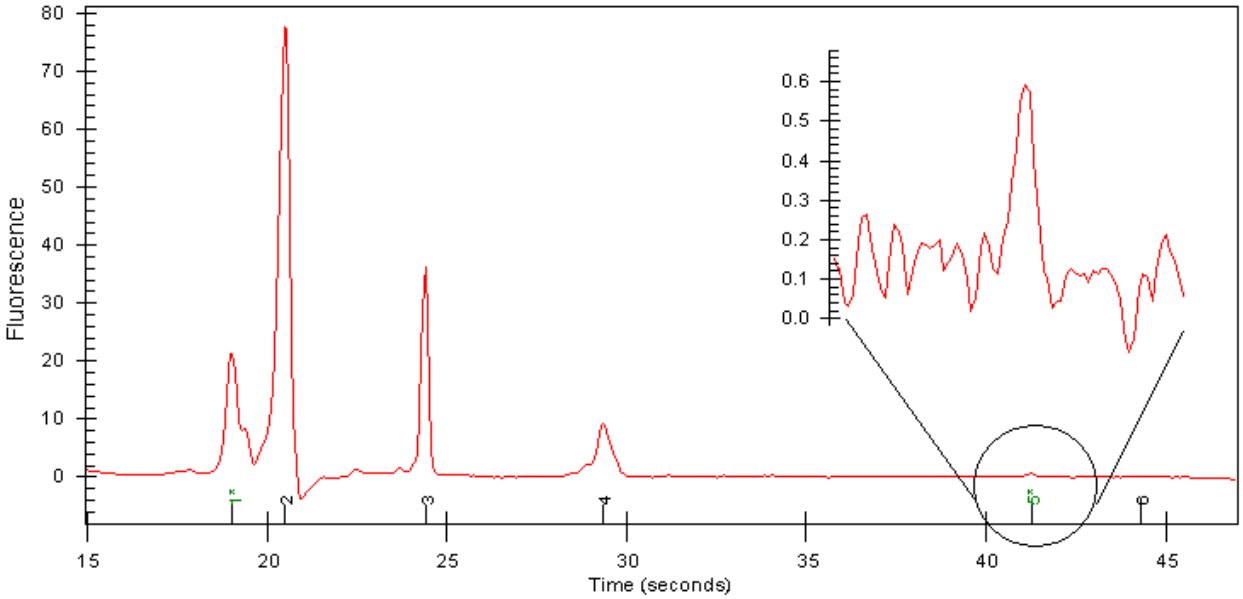
Low or Missing Upper Marker in Ladder



Show me how to solve **Low or Missing Upper Marker in Ladder**

Back to the **Top of List**

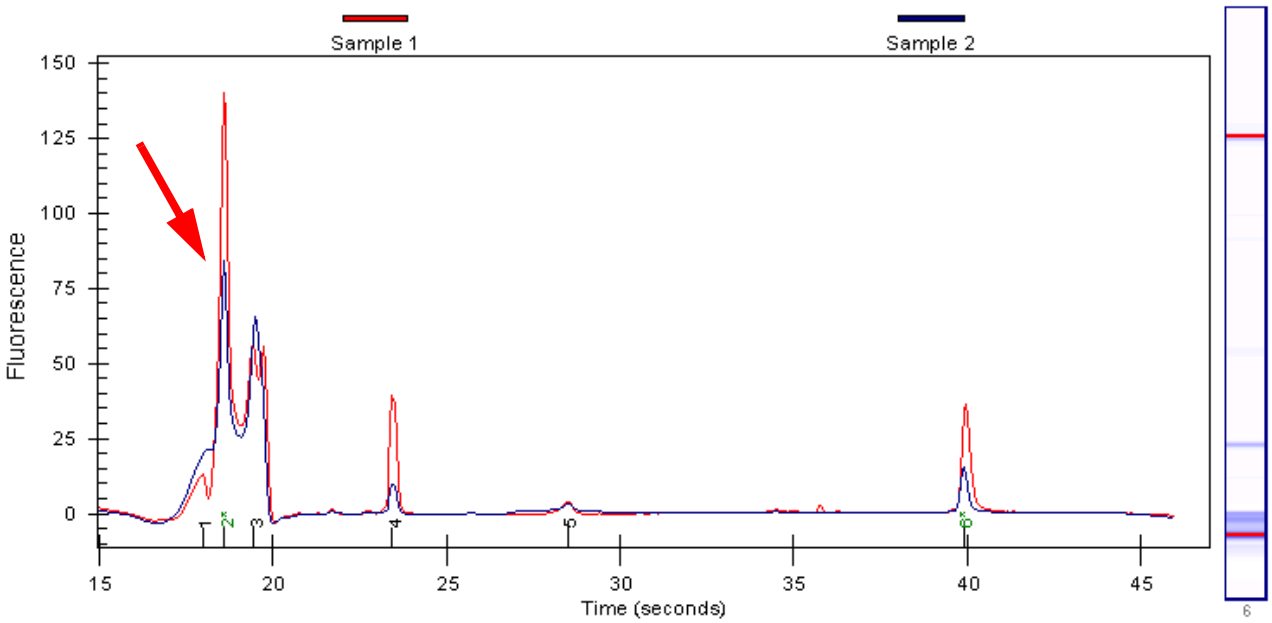
Low or Missing Upper Marker in Sample



Show me how to solve **Low or Missing Upper Marker in Sample**

Back to the **Top of List**

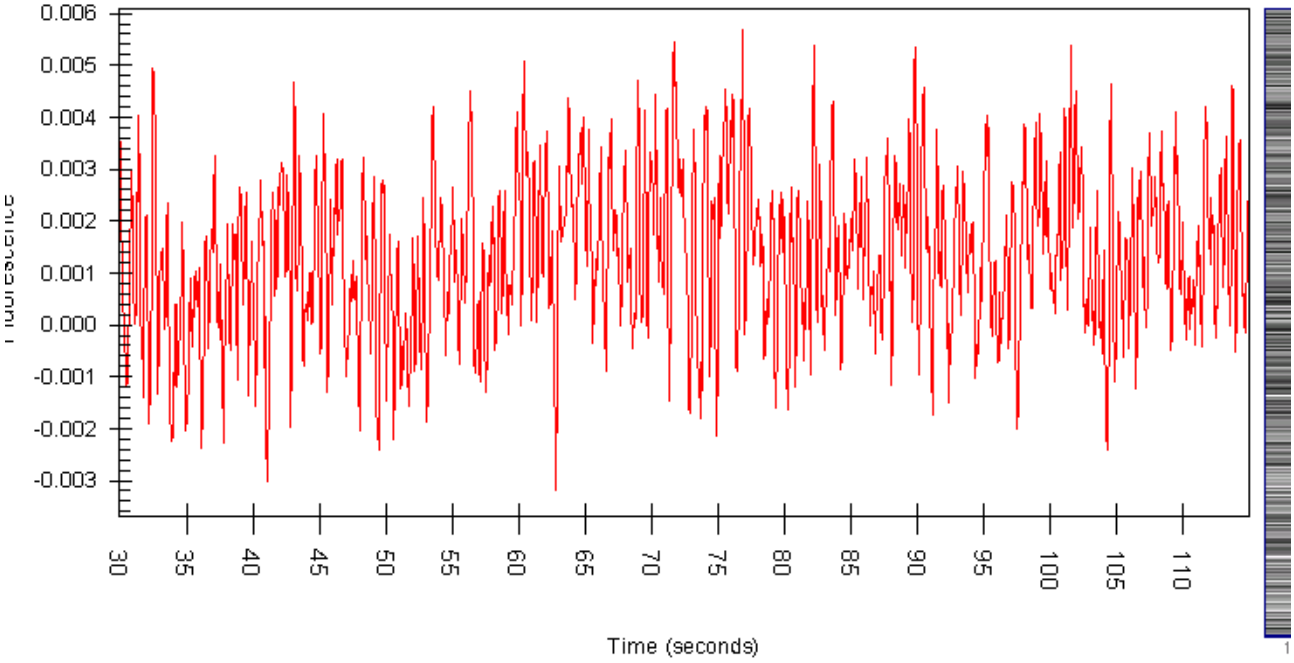
High Lower Marker Variability



Show me how to solve **High Lower Marker Variability**

Back to the **Top of List**

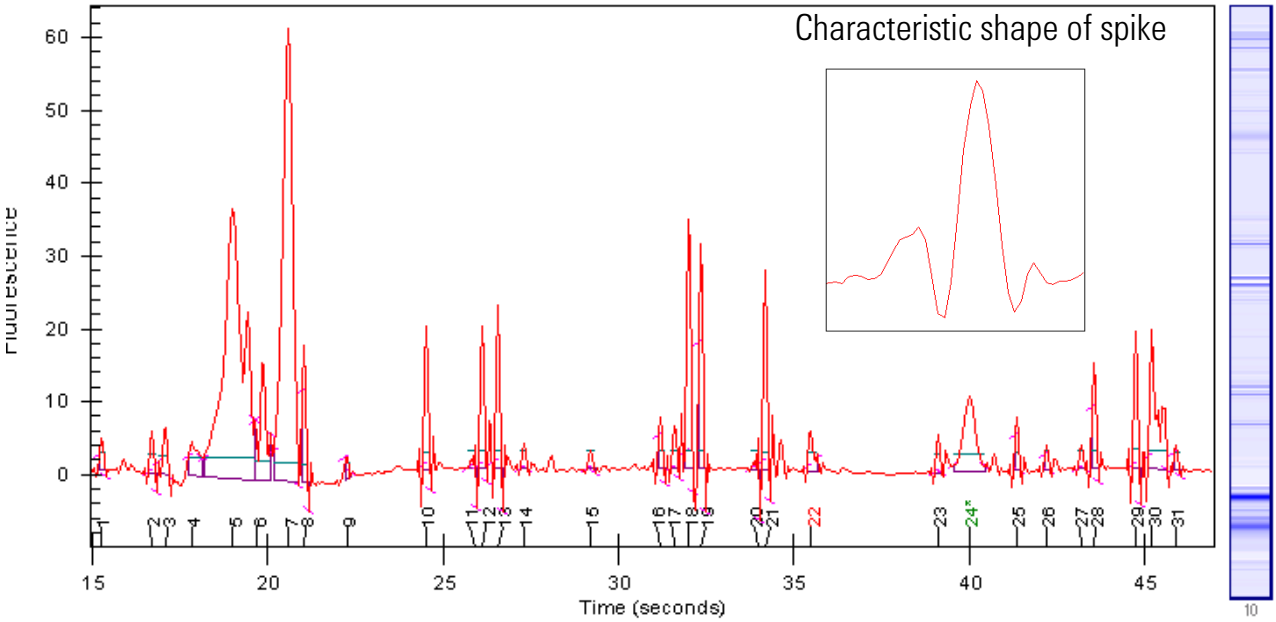
No Peaks



Show me how to solve **No Peaks**

Back to the **Top of List**

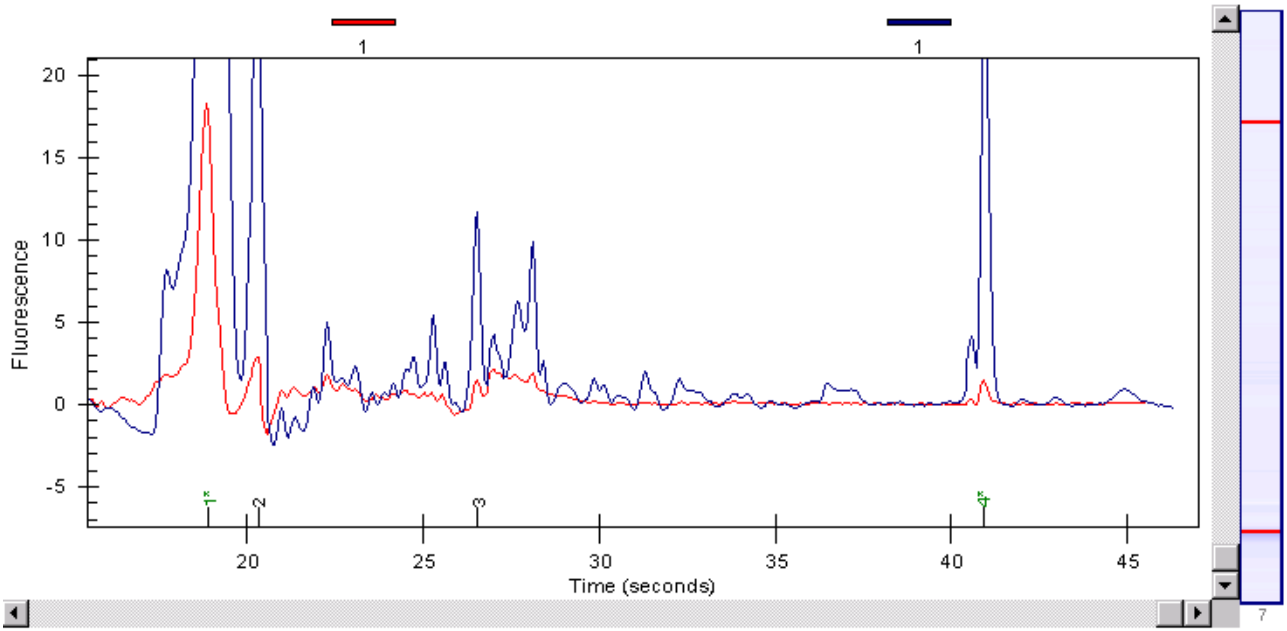
Spikes



Show me how to solve Spikes

Back to the **Top of List**

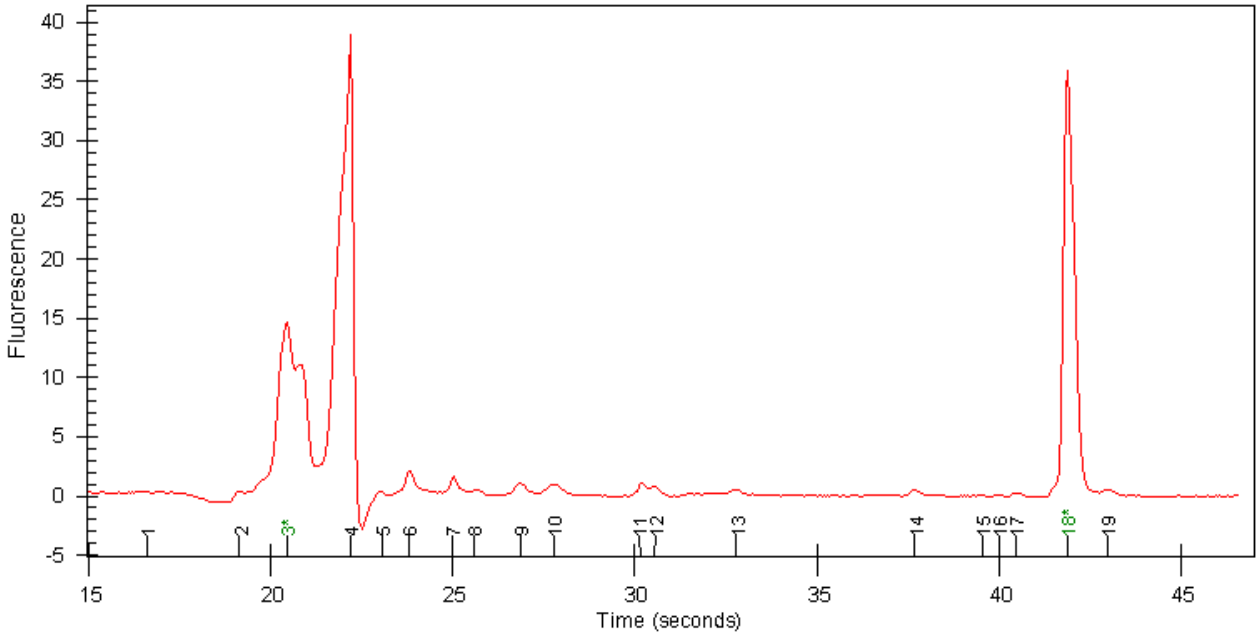
Poor Reproducibility



Show me how to solve Poor Reproducibility

Back to the **Top of List**

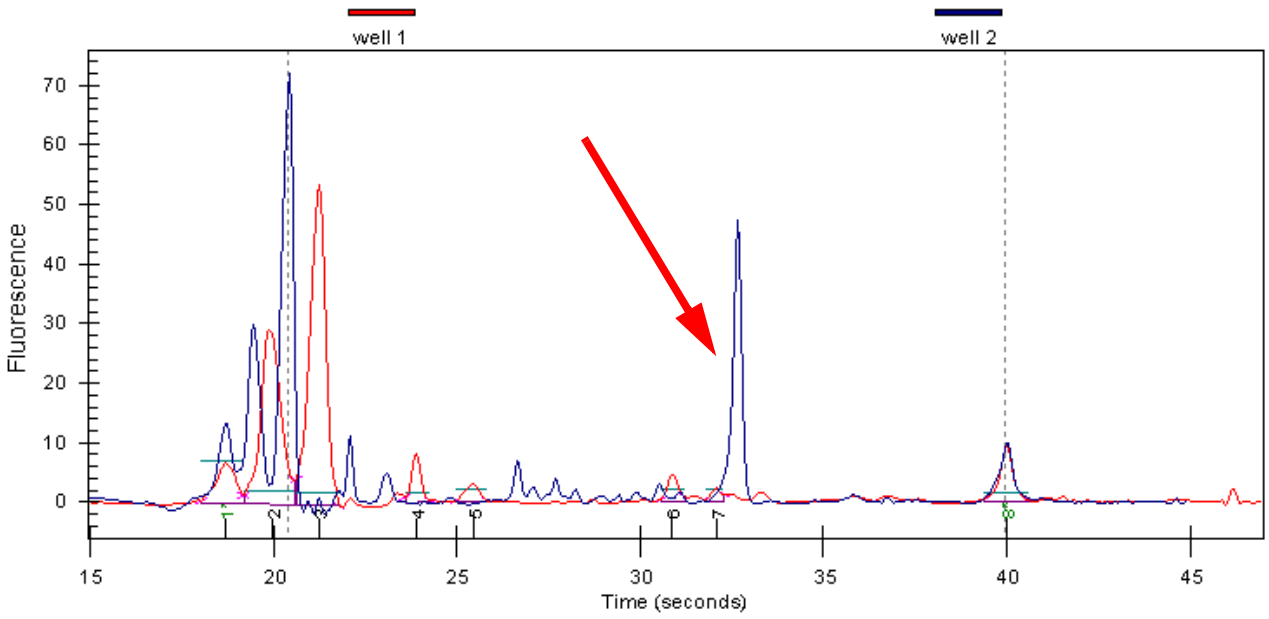
No or Low Sample Peaks



Show me how to solve **No or Low Sample Peaks**

Back to the **Top of List**

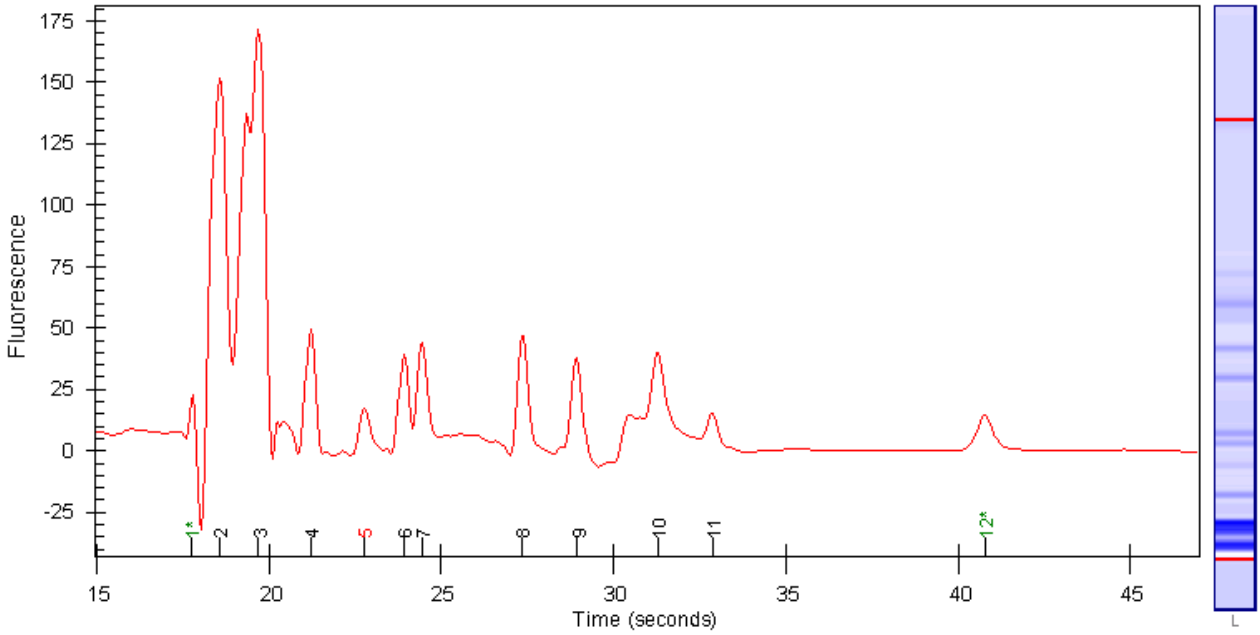
Apparently Missing Sample Peak



Show me how to solve **Apparently Missing Sample Peak**

Back to the **Top of List**

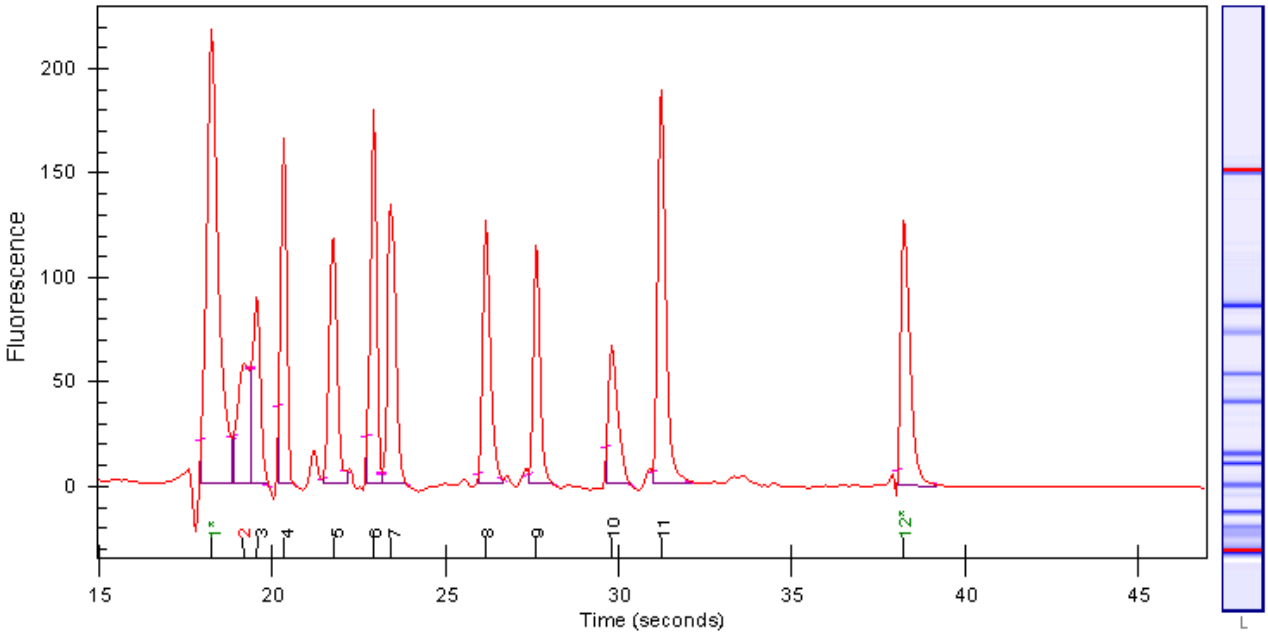
Low Ladder Peaks



Show me how to solve **Low Ladder Peaks**

Back to the **Top of List**

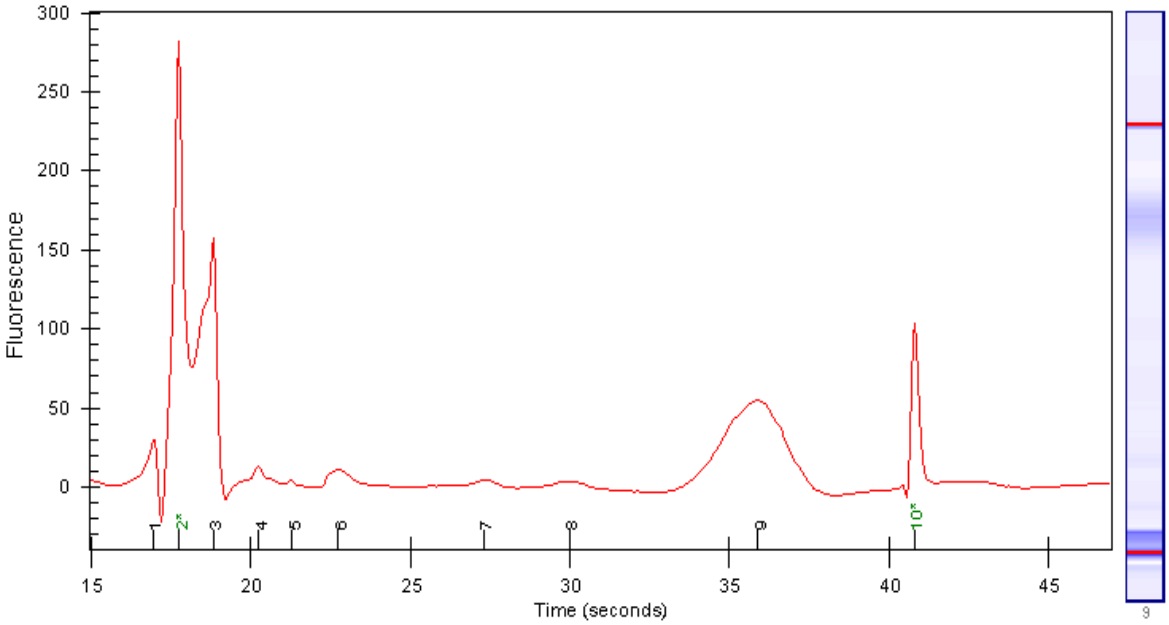
Wrong Alignment of Ladder Peaks



Show me how to solve **Wrong Alignment of Ladder Peaks**

Back to the **Top of List**

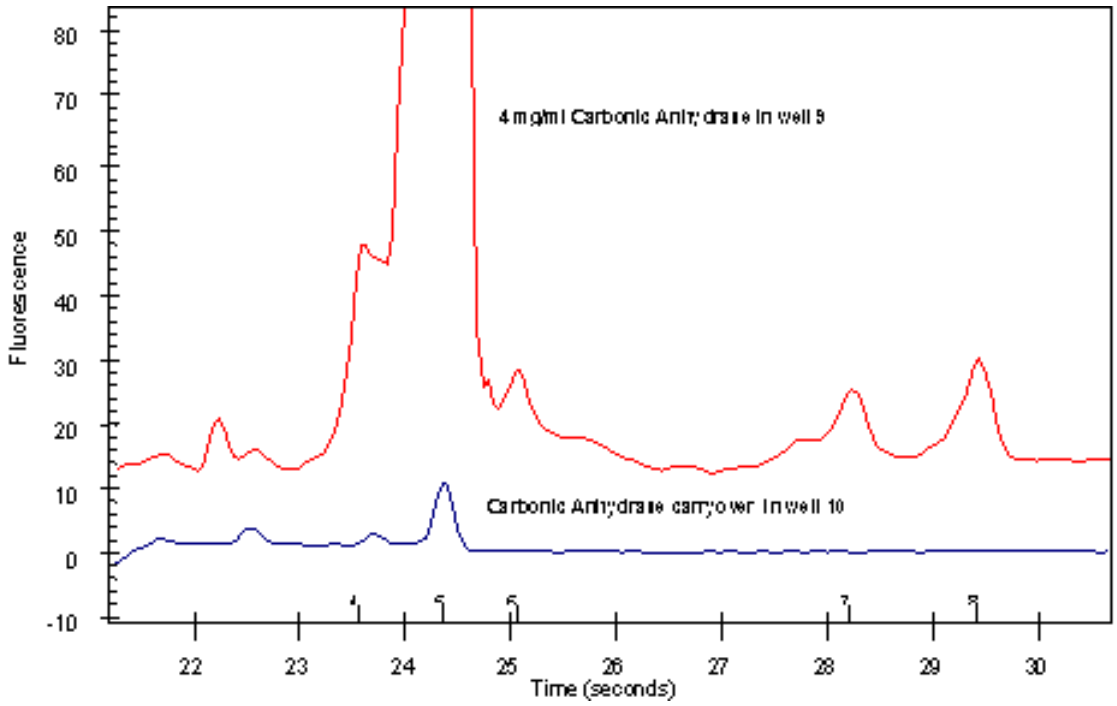
Broad Peaks



Show me how to solve **Broad Peaks**

Back to the **Top of List**

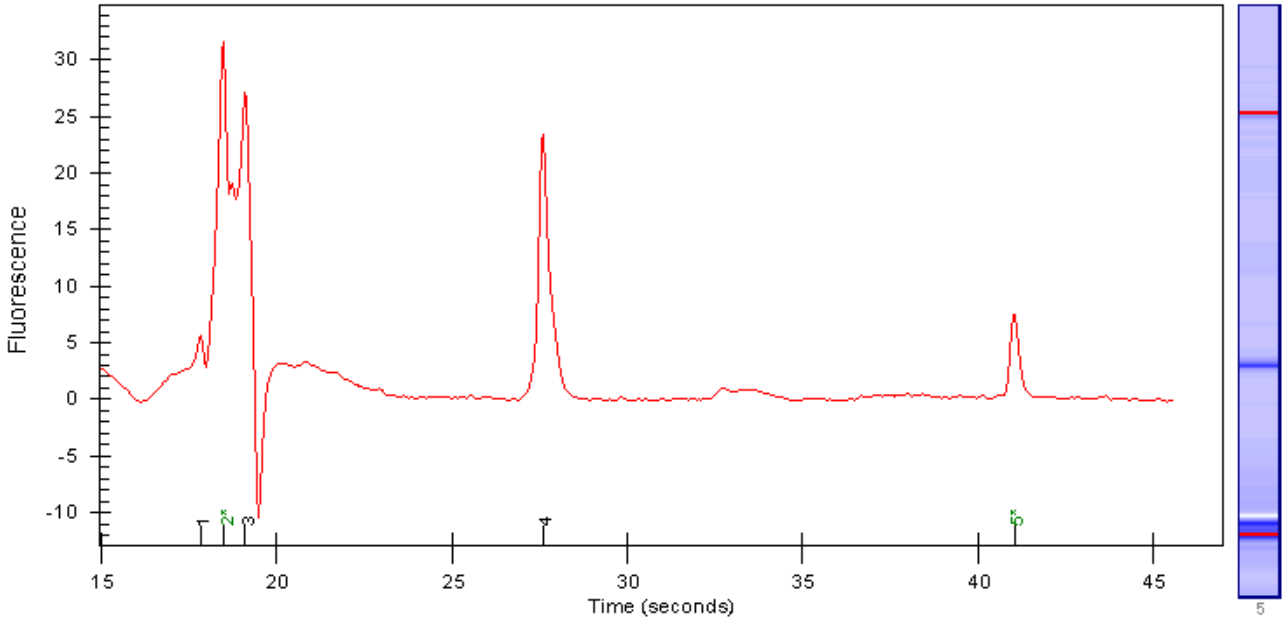
Cross Contamination



Show me how to solve **Cross Contamination**

Back to the **Top of List**

Dips



Show me how to solve **Dips**

Back to the **Top of List**