Agilent Technologies

High Sensitivity Detection Cell for Agilent Capillary Electrophoresis System

General Information

The high sensitivity detection cell increases sensitivity (signal to noise) up to 10-fold over standard 75 µm id capillaries. It comes prealigned in a special optical alignment interface (cell holder) that matches the Agilent CE system.

Part numbers and accessories for the high sensitivity detection cell:

G1600-68713 High Sensitivity Detection Cell Kit

- G1600-60027 High Sensitivity Detection Cell
- G1600-63200 Replacement Fittings
- G1600-60002 Capillary Cassette
- G1600-68715 High Sensitivity Detection Cell Capillary Kit-72

Replacement Capillary Kits

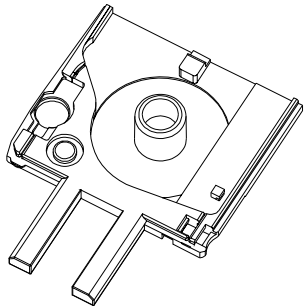
- G1600-68716 High Sensitivity Detection Cell Capillary Kit-56:
  56 cm inlet capillary (75 µm id), and
  8.5 cm outlet capillary (75 µm id)
- G1600-68715 High Sensitivity Detection Cell Capillary Kit-72:
  72 cm inlet capillary (75 µm id), and
  8.5 cm outlet capillary (75 µm id)
- G1600-68714 High Sensitivity Detection Cell Capillary Kit-88:
  88 cm inlet capillary (75 µm id), and
  8.5 cm outlet capillary (75 µm id)

What is Needed?

Older capillary cassettes do not match the new interface design of the high sensitivity detection cell. The new cassettes are modified with a cut-out for fitting, see Figure 1. Please make sure that you have a modified cassette before you continue.

- All buffers, samples and solvents should be filtered through a 0.2 µm filter.
- All vials should be cleaned inside.
- The electrodes, prepunchers and replenishment system should be clean.

Figure 1 **Cut-out on New Capillary Cassette for Fitting High Sensitivity Detection Cell**



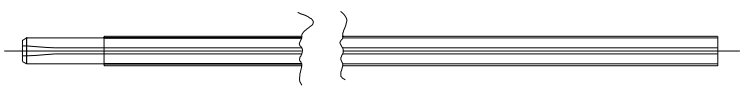
CAUTION

Make sure that you prepare a clean bench for the following procedure. If dust particles enter the capillary or the high sensitivity detection cell, this may lead to poor performance of the cell. Compressed air is very useful for cleaning the parts before they are assembled.

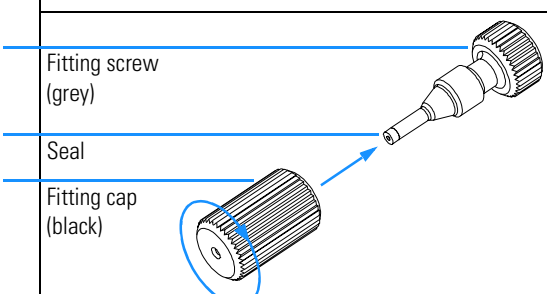
Preparing Capillaries and Fittings for Coupling to High Sensitivity Detection Cell

The capillaries have one end prepared for coupling to the high sensitivity detection cell. The polyimide coating is removed from this end and the edges are bevelled. This end is protected by a cover sleeve upon delivery.

Figure 2 **Prepared End of Capillary (Polyimide Removed)**

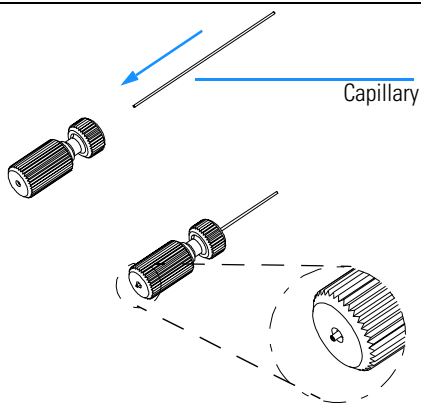


1 Take the fittings and fitting cap out of the bag. Loosely screw the fitting cap onto the fitting screw until you feel it stop. Don't tighten the screw. Purge with compressed air to remove any particles.

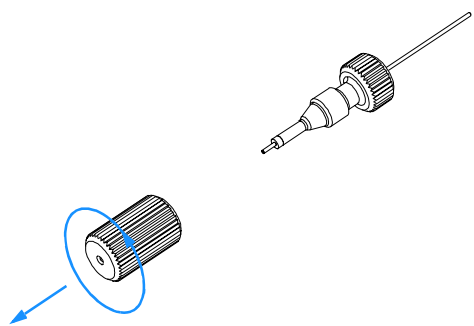


Never slide the capillary through the fitting screw without the fitting cap screwed on. The seal inside the fitting screw could come out, and is difficult to replace.

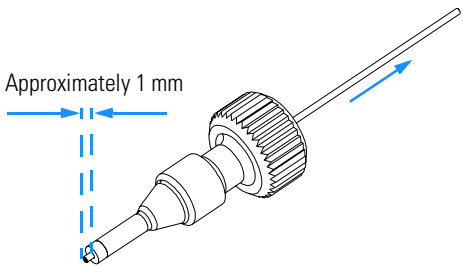
2 Start with the shorter capillary (outlet capillary). Pull the protective cap off the bevelled capillary tip. Only the capillary end with bevelled edges and without polyimide fits correctly to the cell. Do not touch the capillary tip. Push the capillary through the fitting screw until the capillary tip is just visible through the end of the fitting cap.



3 Remove the fitting cap from the fitting screw. Check the capillary tip for particles and remove them if necessary with compressed air. Always store the fitting cap in a bag to protect it from dust.



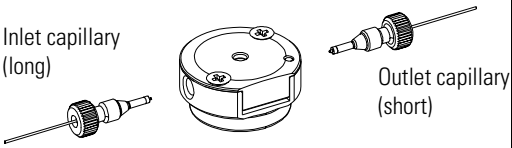
4 Pull the capillary back until it is approximately 1 mm proud of the seal. Don't slide it forward, as this could cause the seal to come out of the fitting.



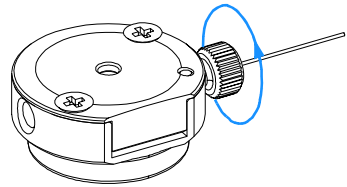
5 Repeat steps 1 to 4 with the inlet capillary. The capillaries and fittings are now ready for coupling to the cell.

Coupling the Capillaries to the High Sensitivity Detection Cell

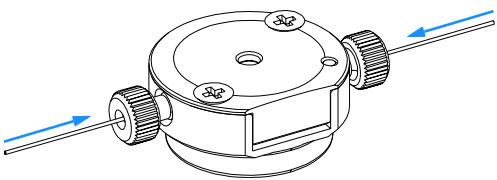
Make sure that you have a clean bench for the following procedure. If dust particles enter the capillary or the cell, this may lead to poor performance of the cell. Also check the ends of both capillaries and fittings for dust particles.



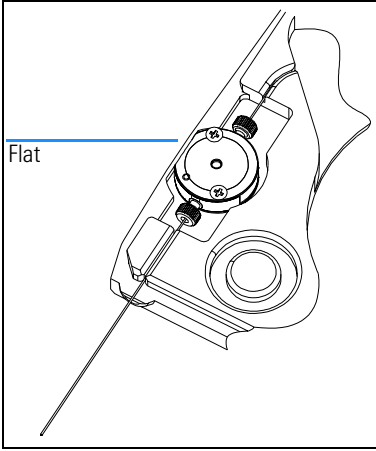
1 Hold the capillary straight and avoid touching the sides of the cell with the capillary tip. Lightly screw the outlet capillary to the cell holder until you feel the stop. Don't tighten the screw yet.



2 Screw the inlet capillary to the cell holder. Hold both capillaries just behind the screw fitting and gently push the capillaries towards each other. Tighten fittings.



3 The cell is now ready for installing into the cassette. Avoid pulling on the capillaries during installation of the cassette in the instrument.

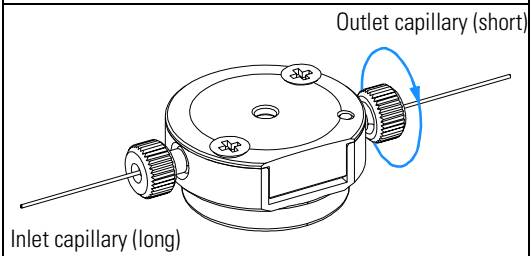


Realigning the Capillaries to the High Sensitivity Detection Cell

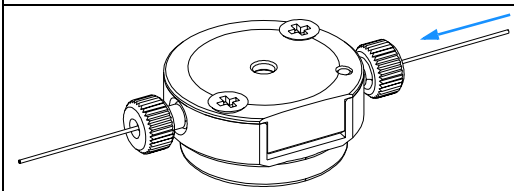
Realigning the capillaries is necessary if one of the problems described in "Troubleshooting" on page 11 occurs.

Make sure that you have a clean bench for the following procedure. If dust particles enter the capillary or the cell, this may lead to poor performance of the cell.

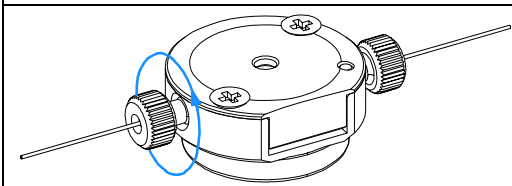
1 Open the outlet fitting screw by turning it approximately 360° counterclockwise.



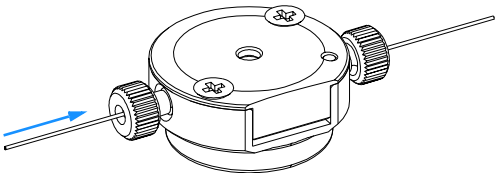
2 Push the outlet capillary gently towards the cell holder. Lightly screw the outlet capillary back to the cell holder. Be sure not to pull the capillary. Don't tighten the screw yet.



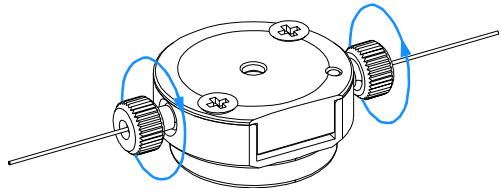
3 Open the inlet fitting screw by turning it approximately 360° counterclockwise.



4 Push the inlet capillary gently towards the cell holder.



5 Screw the inlet capillary to the cell holder. Tighten the fittings.



The high sensitivity detection cell is now ready for installing into the cassette.

NOTE: Avoid pulling on the capillaries during installation into the cassette and instrument.

Storing the High Sensitivity Detection Cell

Short term

- Leave the capillaries connected to the high sensitivity detection cell.
- Put a buffer vial on the inlet and the outlet capillary.

Long term

- For standard and coated capillaries leave them connected to the high sensitivity detection cell.
- Place a vial filled with water at the inlet and flush for 15 minutes.
- Place an empty vial at the inlet and flush for 15 minutes. That will dry the capillary and the high sensitivity detection cell with air.
- For CEC capillaries remove the CEC capillary first and store according the care and use sheet.
- Flush the high sensitivity detection cell with water to remove the buffer.
- Dry the high sensitivity detection cell with air.

The high sensitivity detection cell should be stored in a clean environment (e.g. the plastic container in which the cell is shipped).

Troubleshooting

If, after installation of the High Sensitivity Detection Cell Kit you have problems like those shown in Figure 3 and Figure 4, there are several procedures you can try to get the system running properly.

Figure 3 **Signal Trace Showing Abnormal Baseline—Indicates Air**

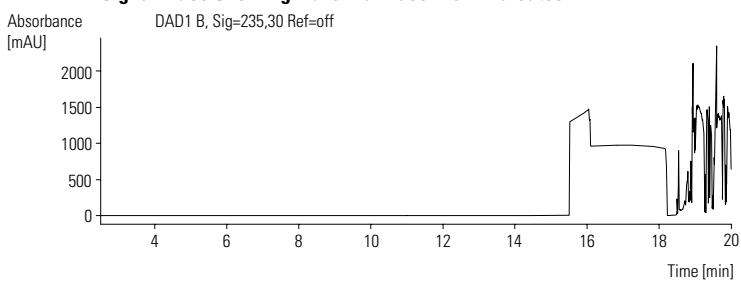
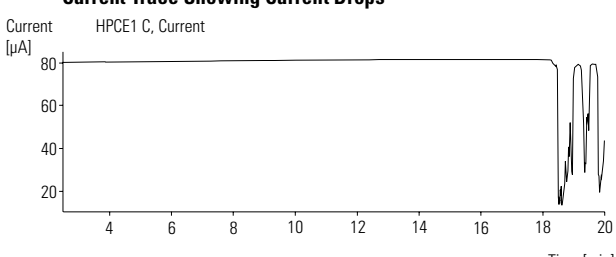


Figure 4 **Current Trace Showing Current Drops**



If you encounter one or several of the following problems:

- baseline jumps in the UV-signal due to coupling problems,
- increased baseline noise due to insufficiently filtered buffers, samples or dirty vials,
- current breakdown when high voltage is applied,
- no current,
- leak current (indicated by yellow bar (current) in graphical user interface),
- current trace showing current drops due to air bubbles in the cell,
- buffer leaks due to improperly coupled capillaries.

Then one of the following may have occurred:

- the capillaries are not properly coupled to the high sensitivity detection cell,
- air bubbles are in the cell,
- particles (from a dirty workbench/vials or from a broken capillary tip) are in the cell,
- the cell is not properly cleaned.

If particles enter the high sensitivity detection cell, this may lead to air bubbles and/or increased baseline noise. As the air bubbles grow, you will observe large baseline drifts or jumps and current drops (see Figure 3 and Figure 4).

CAUTION

Make sure you have a clean bench when you couple/decouple the high sensitivity detection cell assembly. Store the capillaries with the protection cap attached and the cell in the dust-protected box.

Loose fittings can lead to dead volumes at the capillary/cell interface and thus generate band broadening. Also air bubbles may enter the high sensitivity detection cell. Tighten the fittings to the cell holder.

Steps to solve these problems:

1 Perform the realignment.

2 Use degassed buffer. Look for air bubbles in buffer and sample vials.

3 Flush the high sensitivity detection cell and capillary for a longer period of time with running buffer (approximately 10 minutes), then apply 50 mbar pressure for 5 minutes.

4 Filter buffers and samples with a 2 µm pore-size filter or use buffer prepared under cleanroom conditions.

5 If steps 1 through 4 do not improve the situation, flush with 1 N NaOH at elevated temperature (40–60 °C) for at least 60 minutes followed by a flush with water for 3 minutes. This is also recommended if you encounter high baseline noise after repeatedly running samples which stick to the cell windows. The capillary has to be conditioned again with your running buffer after this procedure.

CAUTION

Do not use step 5 with coated capillaries, packed columns or any other capillary where NaOH is problematic.

6 Use the “Cleaning Procedure for the High Sensitivity Detection Cell” on page 14.

CAUTION

The high sensitivity detection cell and fittings are made out of different materials: fused Silica, PEEK (polyetheretherketone) and FVMQ (fluorosilicone rubber). Do not use solvents that can degrade these materials.

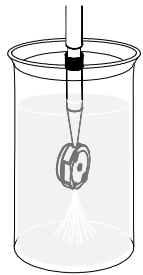
Cleaning Procedure for the High Sensitivity Detection Cell

As in micro-scale technique the operational parts must be protected from contamination with µ-particles. These particles are frequently smaller than can be seen although their effects can be extremely detrimental to the separations. This procedure should ensure that the effects of blockage or contamination with µ-particles can be reversed even in extreme cases.

4.5 ml of HELLMANEX II is included as part of the kit. For replacement, please order 1.3 kg bottle part number 5062-8529.

1. Prepare a 2 % solution (v/v) of HELLMANEX II in capillary electrophoresis grade water (double distilled de-ionized—part number 5062-8578) by adding 1 ml of HELLMANEX II to approximately 50 ml water in a clean glass beaker.
2. Place the cell housing in the beaker and make sure that it is completely immersed in the solution.
3. Flush this diluted solution through the cell via the fitting holes using a 1 ml pipette to ensure maximal wetting of the cell assembly. This procedure will also remove air bubbles from inside the cell. See Figure 5.

Figure 5 **Flushing the High Sensitivity Detection Cell**



4 Place the beaker in an ultra-sonic bath and sonicate for at least 15 minutes. The high sensitivity detection cell housing may change color slightly.

5 Remove the high sensitivity detection cell from the beaker and flush with CE grade water.

6 Place the high sensitivity detection cell in a fresh clean beaker containing CE-grade water and sonicate again for approximately 10 minutes.

7 Remove the high sensitivity detection cell from the beaker and take special care to dry the window area thoroughly. Do not allow water to evaporate from this area as this may deposit a film over the window.

8 Using a microscope check that the windows are clean before recoupling the capillaries to the high sensitivity detection cell.

Troubleshooting Matrix

Table 1 **Troubleshooting Matrix**

What Can Be Seen?	Step 1	Step 2
Current drop, current leaks	Realign	Change fittings, then capillary, use the "Cleaning Procedure for the High Sensitivity Detection Cell" on page 14
Excess baseline noise	Flush with buffer	Use the "Cleaning Procedure for the High Sensitivity Detection Cell" on page 14
Tailing peaks	Realign	Change inlet capillary
Baseline jumps	Flush with buffer for 10 minutes	Realign then use the "Cleaning Procedure for the High Sensitivity Detection Cell" on page 14

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