

This User Guide contains product information on the following LabChip Kits:

HT Protein Express

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Specifications

Sizing Range	P100 Assay: 14 kDa – 100 kDa P200 Assay: 14 kDa – 200 kDa
Sizing Resolution¹	± 10% difference in molecular weight
Sizing Accuracy	± 20%
Linear Concentration Range	5.0 – 2000 ng/μL
Maximum Total Protein Concentration	10 mg/mL
Relative Quantitation	30% CV up to 120 kDa. Above 120 kDa, quantitation is not specified.
Chip Lifetime²	400 samples
Sample Capacity per Chip Prep	400 samples

Sample Conditions

Buffers, Salts and Additives	Please refer to the list in Appendix A for compatibility with specific buffers, salts and additives. If your conditions are not listed, please contact Caliper Life Sciences at 1-877-LABCHIP for more information on compatibility.
Particulates	Sample plates containing particulates or debris should be spun down prior to analysis. All buffers should be filtered with a 0.22 μm cellulose acetate filter.
Salt Concentration	Total salt concentration must not exceed 1M.

¹ Resolution is defined as the height of the valley between two peaks to be no more than 50% of the maximum peak height. Actual separation performance can depend on the sample and application.

² Expected chip lifetime is based on use under normal laboratory conditions and adherence to Caliper preparation protocols, sample guidelines and storage conditions. Individual laboratory results may vary.

Kit Contents

Item	Vial	Quantity
Protein Dye Solution	Blue	1 vial of 0.090 mL
Sample Buffer	White	2 vials, 1.4 mL each
Protein Gel Matrix	Red	2 vials, 1.8 mL each
Protein Ladder	Yellow	1 vial, 0.08 mL
Lower Marker	Green	1 vial, 0.5 mL
Wash Buffer	Purple	4 vials, 1.8 mL each
Spin Filters	—	8 spin filters
Ladder Strip Wells	—	4 spare strip wells
Centrifuge tubes	—	5, 2.0 mL centrifuge tubes
Detection Window Cleaning Cloth	—	1 clean room cloth
Swab	—	3
Buffer Strip Wells	—	4 spare strip wells

Safety Warnings and Precautions

! WARNING ! For Research Use Only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

CAUTION We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

! WARNING ! Dye Solution contains DMSO. Avoid contact with skin and eyes.

! WARNING ! Dye Solution contains SDS. Avoid inhalation and contact with skin and eyes.

! WARNING ! Wash Buffer and Sample Buffer contain LDS. Avoid inhalation and contact with skin and eyes.

! WARNING ! Gel Matrix contains Methylurea. Avoid contact with skin and eyes.

LabChip Kit Guidelines

To ensure proper assay performance please follow the important handling practices described below. Failure to observe these guidelines may void the LabChip Kit product warranty.

NOTE: It is important to keep particulates out of the chip wells, channels and capillary. Many of the following guidelines are designed to keep the chips particulate free.

General

- For assay and instrument troubleshooting, refer to the LabChip HT Software Help file or the Troubleshooting section at <http://www.caliperls.com/products/labchip90.html>, or call 1-877-LABCHIP.
- Allow the chip, sample plate and all reagents to equilibrate to room temperature before use (approximately 20 minutes).
- Clean the chip priming station weekly and the electrodes daily using the cleaning chip provided in the instrument accessory kit. Avoid use of laboratory wipes on chip wells, the priming station, or electrodes. Refer to the Instrument Users Guide Maintenance and Service section for procedures.
- Avoid use of powdered gloves. Use only non-powdered gloves when handling chips, reagents, sample plates and when cleaning the instrument electrodes and electrode block.
- Calibrate laboratory pipettes regularly to ensure proper reagent dispensing.
- Use only the Caliper-supplied clean room cloth on the chip detection window. Use of other, non-approved wipes may leave fluorescent debris which can cause erratic focusing.
- Do not use transfer pipettes or other liquid handling tools that can contaminate liquids with fibers or other debris.
- Water used for reagents and chip preparation procedures must be 0.22-micron filtered deionized or molecular biology grade.

Reagents

- Store reagents at 4 °C when not in use.
- Protect the dye, gel-dye mixture and marker from light. Store in dark and at 4 °C when not in use.
- The gel-dye mixture expires three weeks after preparation.
- For optimal performance, use one reagent kit per chip. The HT Protein Express Reagent Kit contains the reagents to run three 96-well plates or three chip preparations, whichever comes first.

Chips

- New protein chips should be stored refrigerated prior to first use.
- After running the first set of samples, protein chips must be stored at room temperature and used within 30 days.
- Keep the chip detection window free of fingerprints, debris and smudges
- Do not submerge the chip in any solution.
- The sipper must be kept wet at all times and should not be exposed to air for longer than one minute.
- Use care in chip handling to prevent sipper damage. Damage to the sipper can result in inconsistent sampling.
- Avoid exposing the chips to dust by keeping them in a closed environment such as in the chip container or in the instrument before and after chip preparation.
- Chips can be prepared and left in the instrument for extended periods of time, so that samples can be run as needed throughout the day. Caliper recommends the chip be re-prepared after it has been idle for 8 hours, but the chip can be used continually over an 8-hour work day as long as the maximum recommended idle time of 8 hours and total chip lifetime of 400 samples are not exceeded.

Samples

- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow. Sample plates containing gas bubbles and/or particulate debris should be spun down at 3000 rpm (1250 rcf) prior to analysis.
- Recommended well plate volume is 40 μ L for V-bottom 96-well plates. For U-shaped or flat-bottomed well plates, more sample volume is required to achieve a particular fluid depth to ensure the sipper is immersed sufficiently in the sample.
- Up to 4 96-well plates (400 samples) can be run on a single chip preparation. However, full plate analysis is not required. Partial plates (a few wells or rows) can be analyzed as well.

Preparation Procedures

1. Additional Items Required

- 0.6 mL centrifuge tubes and/or 96-well plates for denaturing protein samples

NOTE: Avoid using non-stick variety of lab consumables, which may induce unexpected, or erratic, assay results caused by surface treatments leaching into dye or gel components.

- Reducing agents: BME (beta-mercaptoethanol) or 1M DTT (dithiothreitol)
- Deionized Water: Molecular biology grade or better, 0.22-micron filtered
- 70%-isopropanol solution in DI water

2. Preparing the Gel-Dye and Gel Destaining Solutions

NOTE: It is important that the dye is thawed, fully in solution, and at room temperature before using.

Step	Action
1.	Vortex the HT Protein Express Dye Solution (blue cap) for 10 seconds.
2.	Transfer 520 μL of the HT Protein Express Gel Matrix (red cap) and 20 μL of HT Protein Express Dye Solution (blue cap) to a 1.7 mL centrifuge tube provided with the reagent kit.
3.	Vortex the solution until it is well mixed (approximately 10 seconds). Transfer the solution into one of the spin filters provided with the reagent kit.
4.	Transfer 250 μL of the HT Protein Express Gel Matrix (red cap) into a separate spin filter. This will be used as the destaining solution.
5.	Centrifuge the solutions in both spin filters at RCF = 9300 (approx 10,000 rpm on an 8.3 cm spin dial rotor) for 5 minutes . Remove the filters in the tubes and store in the dark until the chip is to be prepared.
6.	The volumes of gel-dye and destaining solutions prepared are the required amount for one chip prep. If you are preparing additional solution for future use, store the remaining solutions in the dark at 4 °C and use within 3 weeks.

NOTE: Do not exceed 9300 rcf when filtering gel and gel-dye solutions. Exceeding 9300 rcf will change the properties of the gel.

3. Preparing the Sample Denaturing Solution

Step	Action
1.	Pipette 700 μL of HT Protein Express Sample Buffer (white cap) into a 2.0 mL centrifuge vial.
2.	If samples need to be reduced, add 24.5 μL of BME or DTT.
3.	Vortex for 10 seconds. This volume of sample buffer and reducing agent is sufficient to prepare 96 samples.

4. Preparing the Protein Samples and Protein Ladder

NOTE: Samples can be prepared in either a 96-well plate or in microcentrifuge tubes (and subsequently pipetted into a well-plate). Procedures for both are described here.

Step Action

1. For each sample to be analyzed, pipette **7 μL** of denaturing solution into the wells of a 96-well plate or into individual 0.6 mL microcentrifuge tubes.
2. Pipette **2 μL** of each protein sample into the wells of the 96-well plate or microcentrifuge tube. When finished, cover the plate with foil to minimize evaporation. (If running the High Sensitivity Assay, pipette **5 μL** of each protein sample)
3. Allow the HT Protein Express Ladder to warm up to room temperature for 10-20 minutes followed by vortexing for 10 seconds. Pipette **15 μL** of HT Protein Express Ladder into a 0.6-mL centrifuge tube. *Do not add denaturing solution to the ladder.*
4. Heat the ladder tube and sample plate/tubes to **100 °C for 5 minutes**. Do not heat longer or excessive evaporation may occur.
5. Tap or spin the sample plate to move the fluid to the bottom of the wells.
6. Spin the ladder (and sample tubes if used) for 15 seconds using a mini-centrifuge.
7. Add **150 μL** of water to the ladder tube. Vortex the ladder mixture for a few seconds to achieve good mixing.
8. Add **35 μL** of water to each sample well or sample tube. This step should not be done more than an hour before starting the assay. (If running High Sensitivity scripts, add **32 μL** of water to each sample well or sample tube.) Vortex the sample tubes (if used) for a few seconds. If using a plate, a pipettor or plate shaker can be used to mix the water with the samples.
9. If the samples are prepared in tubes, load **40 μL** of each sample onto a 96-well plate.
10. Spin the sample plate to eliminate bubbles and move the fluid to the bottom of the wells.
11. Place the sample plate onto the instrument's plate holder. Align Well A1 of the plate with the label 'A1' on the plate holder.
12. Pipette **150 μL** of protein ladder into Well A of the ladder well strip (conical wells, see Figure 1a).
13. Insert the ladder strip well into the groove marked "L" on the instrument's plate holder. Well A of the strip well should be located closest to the label 'A1' on the plate holder.

Figure 1a: Ladder Strip



Figure 1b: Buffer Strip



5. Preparing the Buffer Strip Well

Step Action

1. Add **200 μL** of HT Protein Express Wash Buffer (purple cap) to each well of the buffer strip (flat-bottomed wells, see Figure 1b).
2. Insert the buffer strip into the groove marked "B" on the instrument's plate holder.

NOTE: Replace the buffer strip with a freshly prepared strip every 8 hours when the chip and instrument is in use.

6. Cleaning the Instrument Electrodes

Step Action

1. Prior to inserting a prepared protein chip into the instrument, Caliper recommends cleaning the instrument electrodes daily. Fill the cleaning chip with 1.2 mL of biology grade water. The cleaning chip can be filled from any well. All wells are connected together by a central U-shaped channel. (The cleaning chip is shipped with the instrument accessory kit).
2. Insert the cleaning chip into the instrument and close the chip holder. This immerses the electrodes in water. Allow for a minimum of 2 minutes of incubation before removing the chip from the instrument. The cleaning chip can stay in the instrument while the protein chip is prepared.

7. Preparing the Protein Chip

Step Action

1. If the chip is new and has been stored in the refrigerator, allow the chip to come to room temperature.
2. Unscrew the four thumbscrews on the Chip Priming Station and remove the top plate.
3. Remove foil cover from chip wells. Use the provided swab dampened with 70% isopropanol to remove residual adhesive on top of wells.
4. Remove the chip from the storage container. Use a pipette tip attached to a vacuum line to dry the top and bottom chip surfaces. DO NOT run the tip over the central region of the detection window. For details on how to set up a house vacuum for aspiration, please refer to Appendix B. It is also recommended to vacuum dry the top plastic plate within the container if the container is to be used for carrying the chip from one location to another.
5. Place the chip into the Chip Priming Station. Carefully pass the sipper through the hole. Thoroughly aspirate all fluid from the chip wells using vacuum line. Aspirating with a dispensing pipette can leave used reagents in the chip wells and is not recommended.
6. Each active well (1, 2, 3, 4, 7, 8, 9 and 10) should be rinsed and aspirated twice with biology grade water (See Figure 1 for well locations on chip). During each aspiration step, carefully vacuum all liquid by reaching all the way to the bottom of the well. Perform this procedure even on new chips.
7. Add the gel-dye solution to Wells 3, 7, 8, and 10 using reverse pipetting, as shown in Figure 1 (below). Caliper recommends reverse pipetting to dispense gel, gel-dye and destaining solutions for accurate delivery of these reagents into chip wells and to avoid introduction of bubbles. (See Figure 2 for reverse pipetting procedures.) It is extremely important to avoid introducing bubbles into the wells. Bubbles pulled into the microchannels will block the reagent flow, causing erratic assay behavior. **Please note new protocol that Gel-Dye is also added to well 3 prior to priming the chip. IMPORTANT: The updated protocol is to be used with HT Labchip SW 2.6.1. LC90 users need to download and install "LabChip HT V2.6.1.210-SP1" first and then "LabChip HT V2.6.1..210 SP1 Assay Addition and Update" for LabChip 90 from the Caliper website.**
NOTE: While updating instrument software please DO NOT uninstall manually previous version of software.

Figure 1: Protein Chip Well Layout

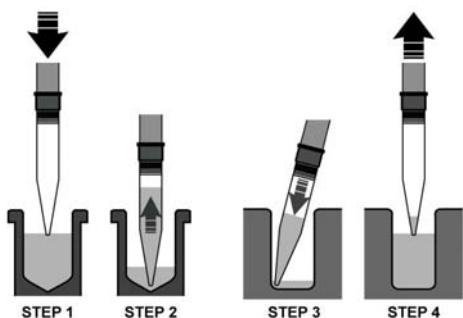
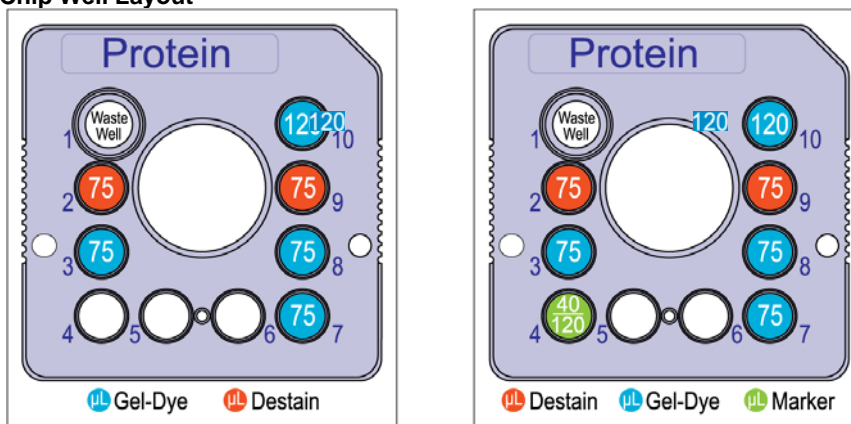


Figure 2: Reverse pipetting technique

- Depress the pipette plunger to the second stop.
- Aspirate the selected volume plus an excess amount from the tube.
- Dispense the selected volume into the corner of the well by depressing plunger to first stop.
- Withdraw the pipet from the well.
- The residual volume can be dispensed back into the tube.

8. Add 75 µL of destain solution to Wells 2 and 9 using reverse pipetting.

9. Priming (Using Manual Priming Station)

- Unscrew the four thumbscrews on the Chip Priming Station and remove the top plate.
- Place the chip into the Chip Priming Station. Carefully insert the sipper into the water reservoir (through the hole).
- Perform the “gel-dye priming” step using the following procedures:
 - a. Pull the plunger of the syringe to the 10.0 mL mark before placing the top plate back on the unit.
 - b. Secure the top plate of the Chip Priming Station over the chip by seating the four thumbscrews (finger tight is sufficient).
 - c. Depress the syringe plunger from the 10.0 mL mark to the 3.0 mL mark and lock in place with the Syringe Clip. Pressurize the chip for **10 minutes**.
 - d. Remove the syringe clip. The pressure within the syringe should return the plunger to approximately the 9.0 mL mark. **DO NOT** pull the plunger out to the 10.0 mL mark, as this could introduce bubbles into the chip, particularly if the sipper is not in contact with the water reservoir.
 - e. Unscrew the four thumbscrews and remove the top plate of the Chip Priming Station.
 - f. Remove the chip from the priming station and place in the chip storage container.

NOTE: Replace and refill the sipper reservoir with fresh water regularly. The sipper reservoir is located on the underside of the chip priming station. Caliper recommends replacing the vial with a new 1.7 mL centrifuge tube monthly.

Priming (Using Automated Priming Station)

- Make sure that the automated priming station is plugged in and the *Ready* status appears on the display.
- Open the priming station by pressing the front lever.
- Make sure that the 0.2 mL tube in the priming station is filled with biology grade water. Refill the tube as needed. If the tube is contaminated, replace it.
- Place the chip in the automated priming station and close the cover. Make sure the cover snaps shut.
- Use the *Pressure* button set the pressure to [**B**].
- Use the *Time* button to set the time. Set the time to setting [**7**] (10 minutes).
- Press Start.
- Once the priming is completed, place one hand on the lid, and with the other hand press the lever to open the priming station.

NOTE: Be sure to periodically clean the O-rings on the top plate of the priming station. Use the provided lint free swab dampened with DI water or 70%-isopropanol solution in DI water to clean the O-rings, using a circular motion. Allow the O-rings to dry before priming a chip.

10. Aspirate the contents of Wells 1 and 4 using vacuum.
11. If the chip will be used to analyze multiple 96-well plates or will be in use for up to 8 hours, add **120 µL** of HT Protein Express Marker (green cap) to Well 4. If the chip will only be used to analyze one 96-well plate or a partial plate and then stored for future use, the marker volume can be reduced to **40 µL**. Make sure the marker volume is pipetted accurately. If there is not enough marker in Well 4, the marker will deplete prior to the completion of the plate. Data collected without marker peaks cannot be analyzed by the software.
12. Remove the chip from the priming station and inspect the chip window. CLEAN both sides of the chip window with the Caliper-supplied cleanroom cloth dampened with a 70%-isopropanol solution in DI water.
13. Insert the chip into the LabChip 90 instrument. Avoid exposing the sipper tip to the air for extended periods of time. Keep the sipper tip immersed in the Buffer Trough whenever possible.
14. Once the chip, sample plate, ladder and buffer strips are loaded onto the instrument, the experiment is now ready to start.

8. Running the HT Protein Express Assay

Step Action

1. Load the LabChip HT software.
2. On the menu bar, select Assay and click on *Protein*. A list of assay choices will appear. Select the appropriate assay script:
 - *HT Protein Express 100*: For sizing of protein in 14kDa to 100 kDa range.
 - *HT Protein Express 100 High Sensitivity*: For sizing of protein in 14kDa to 100 kDa range. Greater sensitivity but requires a larger amount of sample. Slightly lower resolution may be observed.
 - *HT Protein Express 200*: For sizing of protein in 14kDa to 200 kDa range.
 - *HT Protein Express 200 High Sensitivity*: For sizing of protein in 14kDa to 200 kDa range. Greater sensitivity but requires a larger amount of sample. Slightly lower resolution may be observed.
3. Click *Start...* on the upper left corner of the LabChip HT window.
4. The Start menu will open.
 - Enter the *File Prefix* (the name you want your file to be called), *Operator* (your initials), and *ChipID* (the unique ID number on each chip and chip box – e.g., C123A 0499N 04).
 - Select *96 Wells* or *384 Wells*.
 - Click *Start*.
5. The Edit Samples menu will open.
 - Enter *Sample Name*, *Sample Comment*, *Expected Fragments* (the sizes of up to 10 fragments you expect to find in the sample, separated by semi-colons), and +/- % sizing error.
 - Click *OK*.

NOTE: If you are running the High Sensitivity scripts, be sure to make sure the sample dilution ratio is correct, or the quantitation may be off. You can check this by selecting Assay on the menu bar and clicking on HT Protein Express 100/200 High Sensitivity Properties... and selecting the Analysis tab. The dilution ratio for the ladder should be 0.091 and the dilution ratio for the sample should be 0.114.

9. Storing the Protein Chip

At the end of an experiment or an 8-hour day, the chip should be removed from the instrument, cleaned, and stored in the chip container for future use if the chip lifetime has not been reached.

Step Action

1. Remove the reagents from all wells of the chip using vacuum.
2. Each active well (1, 2, 3, 4, 7, 8, 9 and 10) should be rinsed and aspirated twice using biology grade water. During each aspiration step, carefully vacuum all liquid by reaching all the way to the bottom of the well.
3. Fill all chip wells with **120 µL** reagent grade water.
4. Place the chip in its plastic storage container. The sipper should be submerged in the fluid reservoir.
5. Cover the wells with parafilm to prevent evaporation and store the chip at room temperature until next use. The chip must be used to its lifetime (three 96-well plates) within 30 days of analyzing the first plate of samples.

Appendix A – Protein Assay Buffer, Salt and Additive Compatibility

Compatible Buffers, Salts and Additives

Buffer & Salts	Concentration Limit	Additives	Concentration Limit
Tris Chloride	250 mM	Octyl Glucoside	2.5%
Tris Glycine	250 mM	Pluronic F68	0.1%
Hepes	500 mM	Sarcosyl	10%
PBS	8 X	CHAPS	0.5%
Sodium Citrate	150 mM	Tween 20	0.8%
Sodium Phosphate	250 mM	Triton X-100	0.6%
Sodium Acetate	600 mM	SDS	2%
Sodium Chloride	1000 mM	Zwittergent 3-14	0.4%
Sodium Azide	6%	PEG 3350	1%
Sodium Hydroxide	500 mM	Glycerol	30%
Potassium Chloride	900 mM	Urea	8 M
Ammonium Bicarbonate	1000 mM	Sucrose	1 M
Magnesium Chloride	300 mM	DMSO	25%
Imidazole	900 mM	EDTA	100 mM
PhosphoSafe		Ethanol	50%
BugBuster	2.5 X		
BPER			
POP Culture			
Insect POP Culture			

Incompatible Buffers, Salts and Additives

Buffer & Salts	Concentration Limit	Additives	Concentration Limit
RIPA	All	-	-

Appendix B – Chip Well Aspiration Using a Vacuum

Use vacuum to remove fluids from the chip wells.

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, Caliper recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figures 1a and b). To avoid contamination, use a new pipette tip over the permanent tip for each chip aspirated (Figure 2).

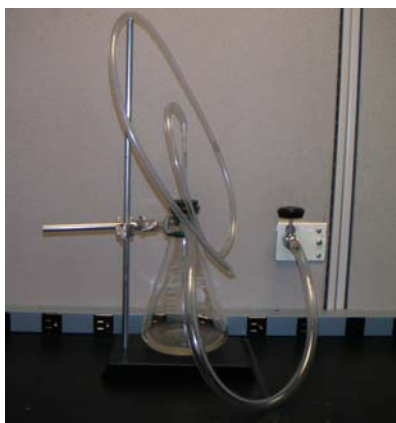


Figure 1a



Figure 1b

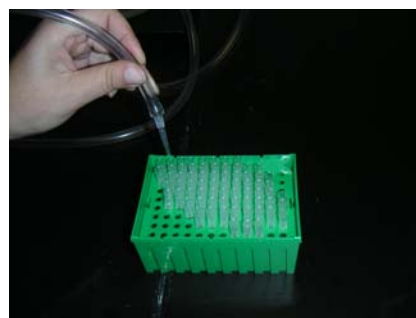


Figure 2

Rinse and aspirate the chip wells prior to priming and storage.

Each active well should be rinsed and aspirated three times using filtered DI water. During each aspiration step, carefully vacuum all liquid by reaching all the way to the bottom of the well. Perform this procedure even on new chips. After priming, be sure to remove any liquid on the tops of the chip wells using a vacuum. Never touch the detection window with anything other than the Caliper-supplied cleanroom cloth.

Reordering Information

Product	Part Number
Protein Express LabChip, Ver 2	Caliper 760499
Protein Express Reagent Kit	Caliper 760328
Buffer Strip Wells	Corning 2593
Ladder Strip Wells	Nunc 248909
2.0 ml Centrifuge Tubes	Eppendorf 022363352

Customer Technical Support

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Fax: 1-508-435-3439

For detailed assay and instrument troubleshooting, refer to the LabChip HT Software Help file or the Troubleshooting section at <http://www.caliperls.com/products/labchip90.html>, or call 1-877-LABCHIP.

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