

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

HT DNA 1K
HT DNA 5K
HT DNA 12K

TABLE OF CONTENTS

HT DNA 1K SPECIFICATIONS	2
HT DNA 5K SPECIFICATIONS	2
HT DNA 12K SPECIFICATIONS	3
SAMPLE CONDITIONS	3
KIT CONTENTS	4
GENERAL	5
REAGENTS	5
CHIPS	5
SAMPLES	6
PREPARATION PROCEDURES	6
1. ADDITIONAL ITEMS REQUIRED	6
2. CLEANING THE INSTRUMENT ELECTRODES	6
3. PREPARING THE GEL-DYE SOLUTION.....	6
4. PREPARING THE LADDER STRIP WELL.....	6
5. PREPARING THE BUFFER STRIP WELLS.....	7
6. PREPARING THE DNA CHIP.....	7
7. RUNNING THE HT DNA ASSAY	9
8. STORING THE DNA CHIP	9
APPENDIX A – CHIP WELL ASPIRATION USING A VACUUM	10
REORDERING INFORMATION	11
CUSTOMER TECHNICAL SUPPORT	11

HT DNA 1K Specifications

Sizing Range	25-1000bp
Sizing Resolution¹	± 5% from 150-600 bp ± 10% from 100-150 bp, 600-1000 bp ± 15% from 25-100 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Linear Concentration Range	0.1 ng/μL-50 ng/μL per fragment
Sensitivity	0.1 ng/μL
Maximum Total DNA Concentration	80 ng/μL total, 50 ng/μL per fragment
Carry-Over	< 0.25%
Quantitation Accuracy	± 30% or ± 1 ng/μL, whichever is greater
Quantitation Precision	20% CV from 25-500 bp, 10% CV from 500-1000 bp
Chip Lifetime²	2000 samples per chip
Number of Samples per Chip Prep	400 samples (four 96-well plates or one 384-well plate)

HT DNA 5K Specifications

Sizing Range	100-5000bp
Sizing Resolution¹	± 10% from 150-500 bp ± 15% from 100-150 bp, 500-1500 bp ± 20% from 1500-5000 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Linear Concentration Range	0.25 ng/μL-50 ng/μL per fragment
Sensitivity	0.25 ng/μL
Maximum Total DNA Concentration	80 ng/μL total, 50 ng/μL per fragment
Carry-Over	< 0.5%
Quantitation Accuracy	± 30% or ± 1 ng/μL, whichever is greater
Quantitation Precision	20% CV
Chip Lifetime²	2000 samples per chip
Number of Samples per Chip Prep	400 samples (four 96-well plates or one 384-well plate)

¹ Resolution is defined as half height or better separation of two peaks. Actual separation performance can depend on the sample and application. Peaks that are resolved less than half height can still be accurately identified by the system software.

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

HT DNA 12K Specifications

Sizing Range	100-12000bp
Sizing Resolution¹	± 10% from 150-1000 bp ± 15% from 1000-2000 bp ± 20% from 2000-8000 bp ± 25% from 100-150 bp, 8000-12000 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Linear Concentration Range	0.25 ng/μL-50 ng/μL per fragment
Sensitivity	0.25 ng/μL
Maximum Total DNA Concentration	60 ng/μL total, 50 ng/μL per fragment
Carry-Over	< 0.5%
Quantitation Accuracy	± 40% or ± 1 ng/μL, whichever is greater
Quantitation Precision	20% CV from 100-5000 bp, 25% CV from 5000-12000 bp
Chip Lifetime²	2000 samples per chip
Number of Samples per Chip Prep	400 samples (four 96-well plates or one 384-well plate)

Sample Conditions

Additives	Caliper recommends that BSA and detergents exceeding 0.05mg/mL and 0.01% (v/v) respectively in concentration not be used. Higher concentrations can result in chip failure. In addition, inorganic and organic solvents are not compatible with the DNA LabChip.
Particulates	Sample plates containing particulates or debris should be spun down prior to analysis. All buffers should be filtered with a 0.22μm cellulose acetate filter.
Salt Concentration	Total salt concentration must not exceed 125mM.
Plasmids	Plasmid concentration in samples must be below 20 ng/μL. Please note that although the HT DNA Assays cannot analyze plasmids, the presence of plasmids above 20 ng/μL can interfere with assay results.

¹ Resolution is defined as half height or better separation of two peaks. Actual separation performance can depend on the sample and application. Peaks that are resolved less than half height can still be accurately identified by the system software.

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

Kit Contents

Item	Vial	Quantity
DNA Dye Concentrate	Blue	1 vial of 0.08 mL
Chip Storage Buffer	White	5 vials, 1.8 mL each
DNA Gel Matrix	Red	3 vials, 1.6 mL each
10X DNA Ladder	Yellow	1 vial, 0.15 mL
DNA Markers	Green	1 vial, 1.5 mL
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 Concentrate contains DMSO. S24/25: Avoid contact with skin and eyes.

Storage: When not in use, store chips and reagents refrigerated at 4 °C. Do not leave chips and reagents unrefrigerated overnight.

LabChip Kit Essential Practices

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.¹

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 LC90 electrodes daily. 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.
- Only the Caliper-supplied clean room cloth can be used on the chip to clean the 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 reagent and chip preparation procedures must be 0.22-micron filtered deionized, molecular biology grade.

Reagents

- Store reagents at 4 °C when not in use.
- The LabChip dye contains DMSO and should be thawed completely before use.
- Gently vortex all kit reagents (except for the HT DNA 12K Marker) before use. (Vortexing may shear large DNA fragments in the HT DNA 12K Marker solution. Instead, mix by gently inverting tube several times.)
- Dispense reagents into chip wells slowly without introducing air bubbles. Insert the pipette tip vertically and to the bottom of the chip well.
- Protect the dye and gel-dye mixture from light. Store in dark at 4 °C when not in use.
- The gel-dye mixture expires three weeks after preparation.

Chips

- New DNA chips should be stored refrigerated prior to first use.
- Once a set of samples has been run, if the chip will be used again in the next 24 hours, it may be left at room temperature. For longer storage periods cover the filled active wells with adhesive foil and store the chip at 4 °C.
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- The sipper must be kept immersed in fluid at all times and should not be exposed to an open environment for long periods of time.
- 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 idle on the instrument for up to 8 hours. This workflow allows analysis of samples as needed throughout the day without having to re-prepare the chip as long as the maximum number of samples per chip prep is not exceeded.

¹ Caliper Life Sciences warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 60 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

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 volume is 30 μ l to 40 μ l for V-bottom 96- or 384-well plates depending on room temperature and level of humidity. For U-shaped or flat-bottomed well plates, higher sample volume will be needed to achieve required fluid depth to ensure the sipper is immersed sufficiently in the sample.
- Up to four 96-well plates or one 384-well plate can be run with a single chip preparation.

NOTE: Caliper recommends diluting high concentration sample (greater than 50 ng/ μ l per fragment or 100 ng/ μ l total) in 1x TE buffer solution.

Preparation Procedures

1. Additional Items Required

- Deionized Water: Molecular biology grade or better, 0.22-micron filtered.
- 70%-isopropanol solution in DI water.
- 10-mL syringe

2. Cleaning the Instrument Electrodes

Step Action

1. Prior to inserting a prepared DNA 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. Remove the cleaning chip and allow the electrodes to air dry for approximately five minutes.

3. Preparing the Gel-Dye Solution

NOTE: It is important that the dye is fully thawed and vortexed before use.

Step Action

1. Transfer **1.0 mL** of HT DNA Gel Matrix (red cap) and **12.5 μ L** of HT DNA Dye Concentrate (blue cap) to the centrifuge tube provided with the reagent kit. Gently vortex the solution until it is well-mixed and spin it down for a few seconds. Transfer the mixture to two spin filters (500 mL each). Label and date the tubes.
2. Centrifuge at **RCF = 9200 for 7.5 minutes**. Store in the dark at 4 °C. Use within 3 weeks.

4. Preparing the Ladder Strip Well

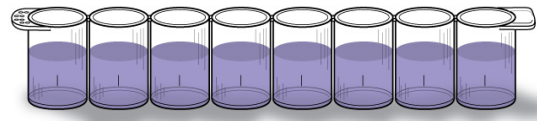
Step Action

1. The DNA Ladder should be prepared in the same buffer as your DNA samples. Add **12 μ L** of HT DNA Ladder to **108 μ L** of 1X DNA buffer solution in a 2.0 mL centrifuge tube. Mix thoroughly by pipetting the solution up and down several times.
2. Add the **120 μ L** solution to Well A of the Ladder Trough (conical wells, see Figure 1a).
3. Insert the Ladder Trough into the groove marked "L". Well A of the strip well should be located closest to the label 'A1' on the plate holder.
4. Place the sample plate onto the instrument's plate holder. Align Well A1 of the plate with the label 'A1' on the plate holder.

Figure 1a: Ladder Strip



Figure 1b: Buffer Strip



5. Preparing the Buffer Strip Wells

Step Action

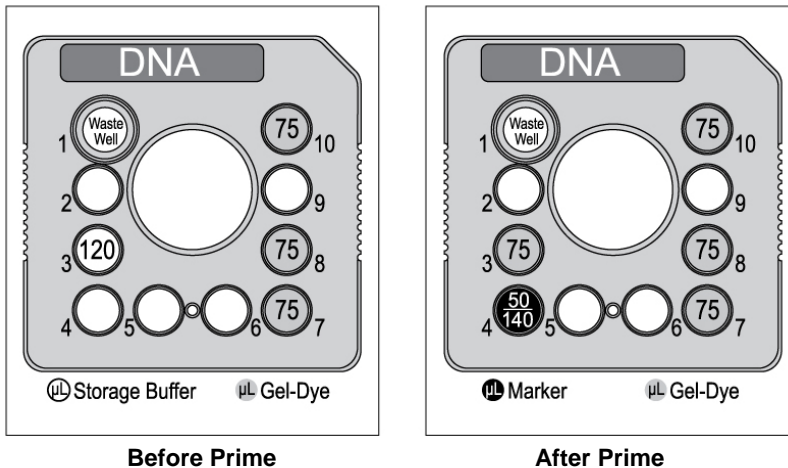
1. Prepare **2.0 mL** of the buffer used in your DNA samples.
2. Add **200 µL** of the buffer to each well of the Buffer Trough (flat-bottomed wells, see Figure 1b).
3. Insert the Buffer Trough into the groove marked "B" on the instrument's plate holder.

6. Preparing the DNA Chip

Step Action

1. If the chip is new and/or has been stored in the refrigerator, allow the chip to come to room temperature.
2. Remove foil cover from chip wells. Use the provided swab dampened with 70% isopropanol to remove residual adhesive on top of wells.
3. 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 A. 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.
4. 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.
5. Each active well (1, 3, 4, 7, 8 and 10) should be rinsed and aspirated twice with biology grade water (See Figure 2 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.

Figure 2: DNA Chip Wells



6. Add **75 µL** of gel-dye solution to Wells 7, 8, and 10 using reverse pipetting (see Figure 3). Caliper recommends reverse pipetting or use of positive displacement pipetting to dispense gel-dye solutions for accurate delivery of these reagents into chip wells and to avoid introduction of bubbles. It is extremely important to avoid introducing bubbles into the wells. Bubbles pulled in the microchannels will block the reagent flow, causing erratic assay behavior.
7. Add **120 µL** of HT DNA Storage Buffer (white cap) to Well 3.

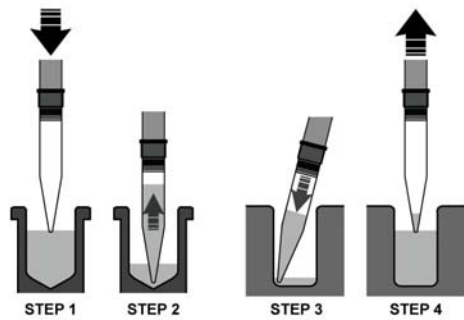


Figure 3: Reverse pipetting technique

- Step 1. Depress the pipette plunger to the second stop.
- Step 2. Aspirate the selected volume plus an excess amount from the tube.
- Step 3. Dispense the selected volume into the corner of the well by depressing plunger to first stop.
- Step 4. Withdraw the pipette from the well.

8. 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** for **HT DNA 1K** chips or **4 minutes** for **HT DNA 5K chips and HT DNA 12K** chips.
 - 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 2.0 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. For the **HT DNA 1K assay**, set the time to setting [**7**] (10 minutes). For the **HT DNA 5K and HT DNA 12K assays**, set the time to setting [**3**] (4 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 3 and 4 using vacuum.
11. Add **75 µL** of gel-dye solution to Well 3 using reverse pipetting.

12. Add HT DNA Marker (green cap) to Well 4. Use **50 µL** for 96-well or **140 µL** for 384-well plate analysis. Please note that the marker well may need to be replenished if the chip is in idle mode on instrument for an extended period of time.
13. 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.
Recommendation: In order to remove any bubbles that may have been introduced during priming, use a 10-mL syringe to apply pressure to Well 4 until a droplet appears at the sipper end.
14. Insert the chip into the LC90 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.
15. Once the chip, sample plate, ladder and buffer strips are loaded onto the instrument, the experiment is now ready to start.

7. Running the HT DNA Assay

Step Action

1. Load the LabChip HT software.
2. On the menu bar, select *Assay* and click on *dsDNA*. A list of assay choices will appear. Select the appropriate assay script:
 - *HT DNA 1K*: For sizing of DNA fragments in 25 to 1000 base pair range.
 - *HT DNA 1K High Resolution*: For sizing of DNA fragments in 25 to 1000 base pair range. Greater resolution with longer analysis time per sample.
 - *HT DNA 5K*: For sizing of DNA fragments in 100 to 5000 base pair range. Fastest analysis time per sample.
 - *HT DNA 12K*: For sizing of DNA fragments in 100 to 12000 base pair range.
 - *HT DNA 12K Extended Time*: To be used only if peaks are cut off using the standard HT DNA 12K script (occurs in some high salt sample buffers).
 - *HT DNA 12K High Resolution*: For sizing of DNA fragments in 100 to 12000 base pair range. Greater resolution with longer analysis time per sample.
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 0377N 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*.

8. Storing the DNA Chip

After use, the chip must be 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, 3, 4, 7, 8, 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. For the manual priming station, add **80 µL** of HT DNA Storage Buffer (white cap) to the same wells. For the automated priming station, add **100 µL** of HT DNA Storage Buffer (white cap) to the same wells.
4. Place the chip in the priming station and follow the same priming procedure described on page 8.
5. Remove the chip from the priming station and place it in its plastic storage container. Add an additional amount of Storage Buffer to well 1. Cover the wells with parafilm if the chip will not be used within 24 hours. Place the chip in its plastic storage container and store in fridge at 4 °C.

Appendix A – 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).

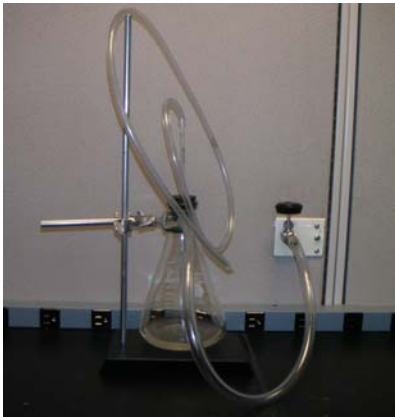


Figure 1a



Figure 1b

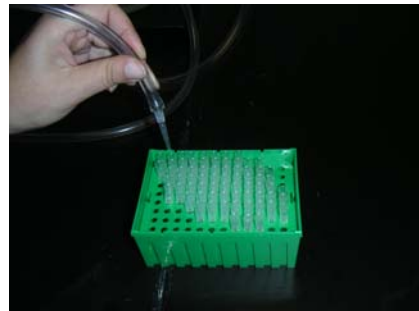


Figure 2

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.

Reordering Information

<u>Product</u>	<u>Part Number</u>
HT DNA 1K/12K LabChip, Version 2	760517
HT DNA 5K/RNA LabChip, Version 2	760435
1K Reagent Kit, ver 2 Only	760526
5K Reagent Kit, ver 2 Only	760566
12K Reagent Kit, ver 2 Only	760569

Customer Technical Support

Caliper Life Sciences
68 Elm Street
Hopkinton, MA 01748-1668
Phone: 1-877-LABCHIP (522-2447)
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.

The chip and reagents supplied with this kit are sold with limited rights of use. The chip may only be used with the specific quantity of reagents supplied with this kit. The purchaser has no right or license to refurbish, reuse, remanufacture, or otherwise use the chip with any other reagents than those specifically supplied in this kit. For more information on the terms and conditions of use of these chips and reagents, please read your AMS90SE™ User Guide or LabChip 90 User Guide. Caliper, the Caliper logo, LabChip, and the LabChip logo are registered trademarks of Caliper Life Sciences.

The reagent kits contain materials manufactured for Caliper by Molecular Probes, Inc., and are provided under a license from Molecular Probes, Inc., for use only in Research, Human Diagnostics, Biohazard Detection, Environmental Testing, Food Testing, Quality Control, and Pathogen Testing.

© Copyright Caliper Life Sciences 2010
<http://www.caliperLS.com>

Doc 450593