Agilent High Sensitivity DNA Kit Quick Start Guide

The complete High Sensitivity DNA Kit Guide can be found in the online help of the 2100 Expert software.

Kit Components

Agilent High Sensitivity DNA Kit (reorder-no 5067-4626)	
Agilent High Sensitivity DNA Chips	**Agilent High Sensitivity DNA Reagents (reorder-no 5067-4627)**
10 High Sensitivity DNA Chips	(yellow) High Sensitivity DNA Ladder
1 Electrode Cleaner	(green) High Sensitivity DNA Markers 35/10380 bp (4 vials)
Syringe Kit	(blue) High Sensitivity DNA Dye Concentrate [1](1 vial)
1 Syringe	(red) High Sensitivity DNA Gel Matrix (2 vials)
	2 Spin Filters (reorder-no 5185-5990)

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Assay Principles

Agilent DNA kits contain chips and reagents designed for sizing and analysis of DNA fragments. Each DNA chip contains an interconnected set of microchannels that is used for separation of nucleic acid fragments based on their size as they are driven through it electrophoretically. Agilent DNA kits are designed for use with the Agilent 2100 Bioanalyzer instrument only.

Applications and Kits

The Agilent High Sensitivity DNA kit is designed for sizing and quantitation of fragmented DNA, DNA sequencing libraries, and DNA samples derived from ChIP.

Agilent DNA kits: DNA 1000 Kit (reorder-no 5067-1504), DNA 7500 Kit (reorder-no 5067-1506), DNA 12000 Kit (reorder-no 5067-1508) and High Sensitivity DNA Kit (reorder-no 5067-4626).

Storage Conditions

- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use to avoid poor results caused by reagent decomposition.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

Agilent Technologies

Equipment Supplied with the Agilent 2100 Bioanalyzer System

- Chip priming station (reorder-no 5065-4401)
- IKA vortex mixer

Additional Material Required (Not Supplied)

- Pipettes (10 µL, 100 µL and 1000 µL) with compatible tips (filter-free, non-autoclaved tips)
- 0.5 mL low-bind microcentrifuge tubes for sample preparation
- Microcentrifuge (> 13000g)

Sample Preparation

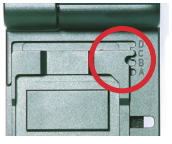
NGS sheared DNA or libraries: For accurate determination of DNA concentration, the total DNA in the samples must be between 100pg/µL to 10 ng/µL.
PCR samples: For accurate determination of DNA concentration, the total DNA in the sample must be between 5 – 500pg/µL.
If concentration of a sample is higher, dilute or use another Agilent DNA assay (DNA 1000, DNA 7500 or DNA 12000).

Specifications

Physical Specifications		Analytical Specifications	
Type	**Specification**	**Specification**	**Agilent High Sensitivity DNA assay**
Analysis time	45 min	Sizing range	50 – 7000 bp
Samples per chip	11	Sizing resolution	50 – 600 bp: ± 10 % 600 – 7000 bp: ± 20 %
Sample volume	1 µL	Sizing accuracy	± 10 %
Kit stability	4 months (see box for storage temperatures)	Sizing reproducibility	5 % CV
Kit size	10 chips 11 samples/chip = 110 samples/kit	Quantitation accuracy[1]	20 %
		Quantitation reproducibility[1]	50 – 2000 bp: 15 % CV 2000 – 7000 bp: 10 % CV
		Quantitative range[1]	5 – 500 pg/µL
		Maximum salt concentration in sample	10 mM Tris and 1 mM EDTA

[1] Determined using the respective DNA ladder as sample

Setting up the Chip Priming Station



1 Replace the syringe:
- **a** Unscrew the old syringe from the lid of the chip priming station.
- **b** Release the old syringe from the clip. Discard the old syringe.
- **c** Remove the plastic cap of the new syringe and insert it into the clip.
- **d** Slide it into the hole of the luer lock adapter and screw it tightly to the chip priming station.

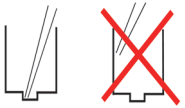
2 Adjust the base plate:
- **a** Open the chip priming station by pulling the latch.
- **b** Using a screwdriver, open the screw at the underside of the base plate.
- **c** Lift the base plate and insert it again in position C. Retighten the screw.

3 Adjust the syringe clip:

a Release the lever of the clip and slide it down to the lowest position.

Essential Measurement Practices

- Handle and store all reagents according to the instructions on the label of the individual box.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 min before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. The dye decomposes when exposed to light and this reduces the signal intensity.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results.



- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 min after preparation. Reagents might evaporate, leading to poor results.
- Do not touch the Agilent 2100 Bioanalyzer during analysis and never place it on a vibrating surface.

Agilent High Sensitivity DNA Assay Protocol

WARNING

Handling DMSO

Kit components contain DMSO. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.

➔ Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

➔ Handle solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Preparing the Gel-Dye Mix

1 Allow High Sensitivity DNA dye concentrate (blue ●) and High Sensitivity DNA gel matrix (red ●) to equilibrate to room temperature for 30 min.

2 Add 15 µL of High Sensitivity DNA dye concentrate (blue ●) to a High Sensitivity DNA gel matrix vial (red ●).

3 Vortex solution well and spin down. Transfer to spin filter.

4 Centrifuge at 2240 g ± 20 % for 15 min. Protect solution from light. Store at 4 °C. Use prepared gel-dye mix within 6 weeks of preparation.

Loading the Gel-Dye Mix

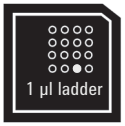
1 Allow the gel-dye mix to equilibrate to room temperature for 30 min before use.
2 Put a new High Sensitivity DNA chip on the chip priming station.
3 Pipette 9 µL of gel-dye mix in the well marked G.
4 Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
5 Press plunger until it is held by the clip.
6 Wait for exactly 60 s then release clip.
7 Wait for 5 s, then slowly pull back the plunger to the 1 mL position.
8 Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.

Loading the Marker

1 Pipette 5 µL of marker (green ●) in all sample and ladder wells. Do not leave any wells empty.

Loading the Ladder and Samples

1 Pipette 1 µL of High Sensitivity DNA ladder (yellow ●) in the well marked ◢.



2 In each of the 11 sample wells pipette 1 µL of sample (used wells) or 1 µL of marker (unused wells).

3 Put the chip horizontally in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
4 Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

Technical Support

Please visit our support web page http://www.agilent.com/genomics/contactus to find information on your local Contact Center.

Further Information

Visit the 2100 Bioanalyzer site at http://www.agilent.com/genomics/bioanalyzer. You can find useful information, support and current developments about the products and the technology.

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