



Agilent DNA 1000 Kit Quick Start Guide

Kit Components

Table 1 DNA 1000 Kit

Agilent DNA 1000 Kit (reorder number 5067-1504)	
DNA Chips	DNA 1000 Reagents (reorder number 5067-1505)
25 DNA Chips	● (yellow) DNA Ladder
1 Electrode Cleaner	● (green) DNA Markers (2 vials)
Syringe Kit	● (blue) DNA Dye Concentrate ¹ (1 vial)
1 Syringe	● (red) DNA Gel Matrix (3 vials)
3 Spin Filters	

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Assay Principles

Agilent DNA kits contain chips and reagents designed for sizing and analysis of DNA fragments. Each Agilent DNA kit 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 only.

Assay Kits

The Agilent DNA 1000 kit provides higher resolution of smaller fragments in comparison of our other DNA sizing kits. It can be used for the following applications:

- Analysis of PCR and RT-PCR products
- RFLP analyses
- Heteroduplex analysis using mismatch cleavage enzymes
- QC of sequencing templates

The complete DNA 1000 kit guide can be found in the online help of the 2100 Expert Software.



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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.

Equipment Supplied with the Agilent 2100 Bioanalyzer

- Chip priming station (reorder number 5065-4401)
- IKA vortex mixer

Additional Material Required (Not Supplied)

- Pipettes (10 µl, 100 µl and 1000 µl) with compatible tips
- 0.5 ml microcentrifuge tubes for sample preparation
- Microcentrifuge

Sample Preparation

- PCR samples: For accurate determination of DNA concentration, the total DNA in sample must be between 0.1–50 ng/µl. If concentration of your particular PCR reaction is excessively high, dilute to 0.1–50 ng/µl.
- Restriction digests: Final concentration of DNA should not exceed 50 ng/µl. Add EDTA and/or heat inactivate the restriction enzyme according to the manufacturer instructions. Restriction endonucleases in combination with non-chelated metal ions may degrade internal DNA markers used in assay kit.

Table 2 DNA 1000 Kit Specifications

Physical Specifications		Analytical Specifications	
Type	Specification	Type	Agilent DNA 1000 Assay
Analysis time	35 min	Sizing range	25 – 1000 bp
Number of samples	12 samples/chip	Sizing resolution	25 – 100 bp: 5 bp 100 – 500 bp: 5 % 500 – 1000 bp: 10 %
Sample volume	1 µL	Sizing accuracy	± 10 %
Kit stability	4 months (see box for storage temperatures)	Sizing reproducibility	5 % CV
Kit size	25 chips 12 samples/chip = 300 samples/kit	Quantitation accuracy ¹	20 %
		Quantitation reproducibility ¹	25 – 500 bp: 15 % CV 500 – 1000 bp: 5 % CV
		Quantitative range ¹	0.5 – 50 ng/µL
		Maximum salt concentration in sample	250 mM for KCl, 15 mM for MgCl ₂ , 250 mM NaCl

¹ Determined using the respective DNA ladder as sample

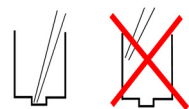
Setting up the Chip Priming Station

- 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 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 DNA dye concentrate (blue ●) and DNA gel matrix (red ●) to equilibrate to room temperature for 30 min.
- 2 Add 25 μL of DNA dye concentrate (blue ●) to a DNA gel matrix vial (red ●).
- 3 Vortex solution well and spin down. Transfer to spin filter.
- 4 Centrifuge at $2240\text{ g} \pm 20\%$ for 15 min. Protect solution from light. Store at $4\text{ }^{\circ}\text{C}$. 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 DNA chip on the chip priming station.
- 3 Pipette 9 μL of gel-dye mix in the well marked .
- 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 .



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 DNA ladder (yellow ●) in the well marked .
- 2 In each of the 12 sample wells pipette 1 μL of sample (used wells) or 1 μL of de-ionized water (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.

