Agilent DNA 1000 Kit Quick Start Guide

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 vial)
1 Syringe	(red) DNA Gel Matrix (3 vials)
3 Spin Filters	

*) "This product is provided under an agreement between Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corporation) and Agilent Technologies. The manufacture, use, sale or import of this product may be subject to one or more of U.S. patents, pending applications, and corresponding international equivalents, owned by Molecular Probes, Inc. The purchaser has the non-transferable right to use the product to detectprotein and/or nucleic acids in microfluidics analysis systems for one or more of the subfields of research, development, quality control, forensics, environmental analysis, biodefense, food safety testing, veterinary diagnostics, or human diagnostics, according to use indicated on the product label or accompanying product literature. For information on obtaining a license, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402-9132. Tel: (541) 465-8300. Fax: (541) 335-0354."

Assay Principles

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

Agilent Technologies

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 in water.
- 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.

Physical Specifications

Type	Specification
Analysis run time	35 minutes
Number of samples	12 samples/chip
Sample volume	1 µl
Kit stability	4 months (Storage temperature see individual box!)

Analytical Specifications

Type	Agilent DNA 1000 Assay
Sizing range	25–1000 bp
Typical sizing resolution	± 5 bp 25–100 bp ± 5 % 100–500 bp ± 10 % 500–1000 bp
Sizing accuracy	± 10 %* (for ladder as sample)
Sizing reproducibility	5 % CV (for ladder as sample)
Quantitation accuracy	20 %* CV (for ladder as sample)
Quant. reproducibility	25-500 bp: 15 % CV; 500-1000 bp: 5 % CV (for ladder as sample)
Quantitative range	0.1–50 ng/µl
Maximum salt	250 mM for KCl or NaCl, 15 mM for $MgCl_2$

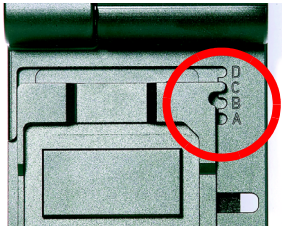
*) Some fragments below 70 bp may deviate from the above specifications.

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 priming station.

2 Adjust the base plate:



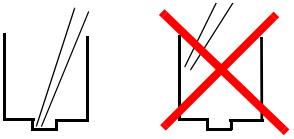
- **a** Open the chip priming station by pulling the latch.
- **b** Using a screwdriver, open the screw at the underside of the base plate.
- **c** Lift the base plate and insert it again in position C. Retighten the screw.

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 reagent and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes 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 minutes 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 1000 Kit Quick Start Guide

Agilent DNA 1000 Assay Protocol - Edition April 2007

WARNING

Handling DMSO

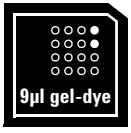
Kit components contain DMSO. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.

Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. Handle the DMSO stock 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. Vortex DNA dye concentrate (blue ●) and add 25 µl of the dye to a 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.

Loading the Gel-Dye Mix

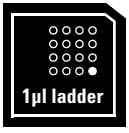


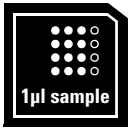
1. Allow the gel-dye mix equilibrate to room temperature for 30 min before use.
2. Put a new DNA chip on the chip priming station.
3. Pipette 9.0 µl of gel-dye mix in the well marked G.
4. Make sure that the plunger is positioned at 1 ml and then close the chip priming station.
5. Press plunger until it is held by the clip.
6. Wait for exactly 60 s then release clip.
7. Wait for 5 s. Slowly pull back plunger to 1ml position.
8. Open the chip priming station and pipette 9.0 µl of gel-dye mix in the wells marked G .

Loading the Markers

1. Pipette 5 µl of marker (green ●) in all 12 sample wells and ladder well. Do not leave any wells empty.

Loading the Ladder and the 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 within 5 min.

Technical Support In the U.S./Canada: 1-800-227-9770 (toll free); lsca-ibs-support@agilent.com. In Europe: call your local Customer Care Center; bio_solutions@agilent.com. In Japan: 0120 477 111; yan_ccr@agilent.com
In Asia Pacific: call your local Customer Care Center; Bioanalyzer_ap@agilent.com

Further Information Visit Agilent Technologies' unique Lab-on-a-Chip web site. It is offering useful information, support and current developments about the products and the technology: http://www.agilent.com/chem/labonachip.

G2938-90015

Part Number: G2938-90015
Edition 04/2007
Printed in Germany

Agilent Technologies
Hewlett-Packard-Straße 8
76337 Waldbronn, Germany