



Agilent DNA 7500 and DNA 12000 Kit Quick Start Guide

Agilent DNA 7500 Kit (reorder number 5067-1506) and Agilent DNA 12000 Kit (reorder number 5067-1508)

DNA Chips	Reagents ¹ & Supplies
25 DNA Chips	● (yellow) DNA Ladder
1 Electrode Cleaner	● (green) DNA Markers (2 vials)
Syringe Kit	● (blue) DNA Dye Concentrate ² (1 vial)
1 Syringe	● (red) DNA Gel Matrix (3 vials)
	3 Spin Filters

¹ DNA 7500 Reagents (reorder number 5067-1507); DNA 12000 Reagents (reorder number 6067-1509)

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Assay Principles

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

Assay Kits

Agilent DNA 7500 and DNA 12000 kits are designed for the sizing and quantitation of double-stranded DNA fragments from 100 to 7500 base pairs (DNA 7500) and 100 to 12000 bp (DNA 12000 respectively). These kits can be used to analyze, for example, PCR and RT-PCR products as well as restriction digests. The complete DNA 7500 and DNA 12000 kit guide can be found in the online help of the 2100 Expert Software.



Agilent Technologies

Agilent DNA 7500 and DNA 12000 Kit Quick Start Guide

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

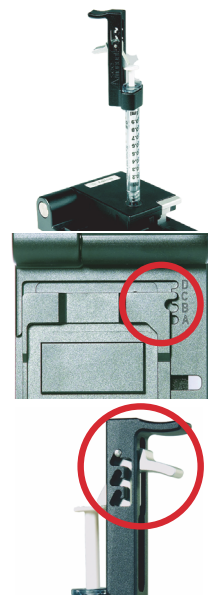
- For accurate determination of DNA concentration, the total amount of DNA in sample must be between 0.5–50 ng/µL. If concentration is excessively high, dilute to 0.5–50 ng/µL.
- When analyzing restriction digests, add EDTA or heat inactivate the restriction enzyme according to the manufacturer's instruction. Restriction endonucleases in combination with non-chelated metal ions may degrade internal DNA marker.

Physical Specifications		Analytical Specifications		
Type	Specification	Type	Agilent DNA 7500	Agilent DNA 12000
Analysis time	30 min	Sizing range	100 – 7500 bp	1000 – 12000 bp
Samples per chip	12	Sizing resolution	100 – 1000 bp: 5 % 1000 – 7500 bp: 15 %	100 – 1000 bp: 5 % 1000 – 12000 bp: 15 %
Sample volume	1 µL	Sizing accuracy	± 10 %	± 15 %
Kit stability	4 months (see box for storage temperatures)	Sizing reproducibility	5 % CV	5 % CV
Kit size	25 chips 12 samples/chip = 300 samples/kit	Quantitation accuracy ¹	20 %	25 %
		Quantitation reproducibility ¹	100 – 1000 bp: 10 % CV 1000 – 7500 bp: 5 % CV	100 – 1000 bp: 15 % CV 1000 – 12000 bp: 10 % CV
		Quantitative range ¹	0.5 – 50 ng/µL	0.5 – 50 ng/µL
		Maximum salt concentration in sample	250 mM for KCl, 15 mM for MgCl ₂ , 250 mM NaCl	250 mM for KCl, 15 mM for MgCl ₂ , 250 mM NaCl

¹ Determined using the respective DNA ladder as sample

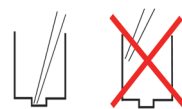
Setting up the Chip Priming Station

- 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 up to the top 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 7500 and DNA 12000 Assay Protocol - Edition April 2007

WARNING

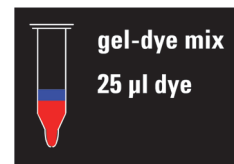
Handling DMSO

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 μ L of the dye to a DNA gel matrix vial (red ●).
- 3 Vortex solution well and spin down. Transfer to spin filter.
- 4 Centrifuge at 1500 g \pm 20 % for 10 min. Protect solution from light. Store at 4 °C.



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 μ L 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 30 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 μ L of gel-dye mix in the wells marked **G**.



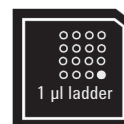
Loading the Marker

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



Loading the Ladder and the Samples

- 1 Pipette 1 μ L of DNA ladder (yellow ●) in the well marked **L**.
- 2 In each of the 12 sample wells pipette 1 μ L of sample (used wells) or 1 μ L 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.

