CE dsDNA 4000 Analysis Kit

Instruction Manual

Catalog Number 148-4132

BIO-RAD

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Section 1
Introduction

The CE dsDNA 4000 Analysis Kit is optimized for use with the BioFocus capillary electrophoresis system and resolves double-stranded DNA fragments ranging in size from 100 to 4,000 base pairs. The kit is useful for a variety of applications including determination of PCR* products, optimizing PCR conditions, and characterizing restriction enzyme digests. High performance capillary electrophoresis is an ideal approach for nucleic acid separations, providing rapid analysis, quantitative information, minimal consumption of sample, and direct detection of resolved fragments without the need for ethidium bromide staining.

High resolution DNA separations are achieved by dynamic sieving** capillary electrophoresis, a technique that incorporates a hydrophilic polymer in the electrophoresis buffer. The polymer-containing buffer is replenished between each analysis, providing reproducibility of migration time and peak area. The analysis employs a BioCAP™ capillary, coated internally with a novel polymer (polyAAEE), insuring high-resolution separations over many runs.

Section 2
CE dsDNA 4000 Kit Components

The CE dsDNA 4000 Analysis Kit contains all the reagents necessary for 40 setups, with up to 30 runs per setup using the BioFocus capillary electrophoresis system.

- Two BioCAP DNA analysis capillaries, 50 cm x 75 µm ID x 375 µm OD, with detection window 8 cm from the end.
- CE dsDNA Run Buffer, containing sieving polymer in 178 mM tris, 178 mM boric acid, 4 mM EDTA, 60 ml
- CE dsDNA Dilution Buffer, 178 mM tris, 178 mM boric acid, 4 mM EDTA, 60 ml
- CE dsDNA 100 bp Ladder, 100 µl at 100 µg/ml in TE buffer, pH 8.0
- Instruction Set

2.1 Capillary Preparation and Use

The BioCAP DNA analysis capillary supplied with this kit is designed for use with the BioFocus user assembled capillary cartridge. For information on capillary installation, refer to the instructions included with the BioFocus cartridge assembly kit, catalog number 148-3050, or BioFocus Cartridge, catalog number 148-3052. For use with this kit, the capillary cartridge can be assembled with 24 to 44 cm (total length) of capillary installed. When using a fresh capillary it is recommended to perform several blank runs to equilibrate the capillary.

2.2 Using the CE dsDNA 100 Base Pair Ladder

The CE dsDNA Ladder is a mixture of 10 double-stranded sequences ranging in length from 100 bp to 1,000 bp in exact 100 bp increments. Under the conditions described below, the standard will exhibit 10 major peaks at 260 nm (see Figure 1). Pipette 20–50 µl of the CE dsDNA Ladder into a 500 µl sample vial. If the sample is going to be used for multiple analysis, a drop of mineral oil can be layered carefully on top of the sample to help prevent evaporation and extend the useful life of the sample. Routinely, the 100 Base Pair Ladder should be stored at 4 °C. However, longer term storage at -20 °C may increase shelf life.

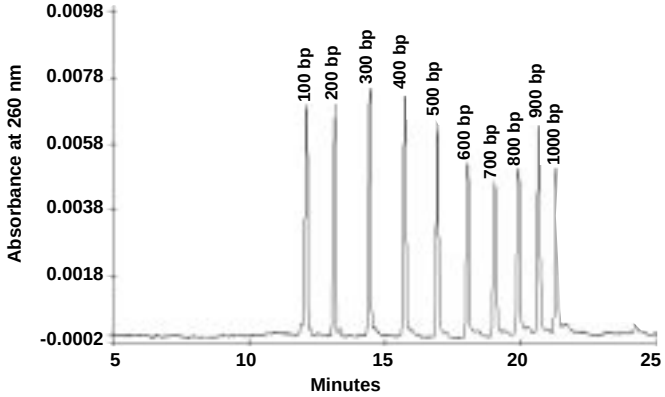


Fig. 1. Electropherogram of CE dsDNA 100bp Ladder (catalog number 148-2019).

2.3 Sample Preparation

In Figure 1 the concentrations of the individual DNA fragments are 10 µg/ml. In general, DNA component concentrations should be in the range of 5 to 20 µg/ml. The presence of salt in the sample can reduce the injection efficiency in electrophoretic injection, resulting in decreased sensitivity. If the salt concentration of the sample is known to be greater than 50 mM, or if peak response is much smaller than expected, the sample may have to be desalted by dialysis or ultrafiltration (see Desalting, Section 5.1).

2.4 Buffer Preparation for Resolving Fragments of 100–1,000 Base Pairs

The CE dsDNA 4000 Run Buffer should be brought up to room temperature before use. All run buffer vials should be filled to capacity to ensure proper contact with the capillary and electrode. Degassing by centrifugation is highly recommended. To degass pipette buffer into 500 µl vials and centrifuge the vials for at least 3 minutes in a microcentrifuge immediately before inserting them into the BioFocus carousels.

2.5 Buffer Preparation for Resolving Fragments of 1,000–4,000 Base Pairs

The dsDNA Run Buffer supplied in this kit gives good resolution of double-stranded DNA from 100–1,000 base pairs using a 24 cm x 75 µm coated capillary cartridge with the separation

parameters described above. This size range can be extended to about 4000 base pairs by diluting the dsDNA Run Buffer 4-fold with the dsDNA Dilution Buffer supplied with the kit. Add 1.0 ml of the dsDNA Run Buffer to 3.0 ml of the dilution buffer and mix for 20 minutes. Figure 2 shows the analysis of a 200 base pair ladder on a 44 cm DNA analysis capillary using the diluted dsDNA Run Buffer, at 3.5 kV.

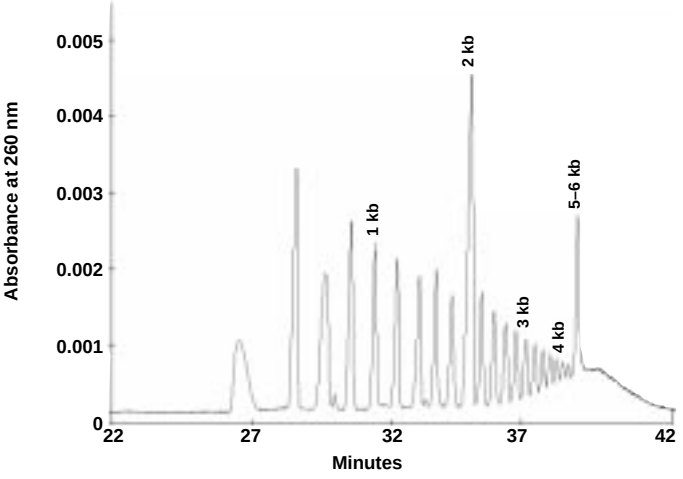


Fig. 2. Electropherogram of 200 base pair ladder (Gensura catalog number SLM-200)

Section 3
Analysis Conditions on the BioFocus System

The cartridge data and instrument configuration are shown in Table 1 and the dsDNA analysis method for use with 24 cm capillaries in Table 2. A typical shutdown method is depicted in Table 3.

3.1 Configuration

The configuration specified in Table 1 includes sufficient reagents to do up to 30 analysis and a clean up of the capillary at the end of the automation sequence. The reagents must be assigned to carousel positions with vial holders that can accommodate the vial size recommended in Table 1.

Table 1. BioFocus Configuration for dsDNA Analysis

ID: DNA Description: dsDNA Analysis

INLET CAROUSEL POSITIONS

Pos	Type	ID/Description	Contents	Vial Size	Amount in Vial
1	R	DNA_RUN	Run buffer	500 µl	500 µl
2	R	DNA_PREP	Run buffer	500 µl	500 µl
3	R	WATER_DIP	Water	500 µl	500 µl
9	R	WATER/Shutdown	Water	500 µl	500 µl
10	R	NITROGEN/Shutdown	Empty	500 µl	
11	S	100 bp ladder	Sample	500 µl	20–50 µl

OUTLET CAROUSEL POSITIONS

Pos	Type	ID/Description	Contents	Vial Size	Amount in Vial
1	R	DNA_RUN	Run buffer	500 µl	500 µl
2	R	WATER/Shutdown	Water	500 µl	500 µl
32	W	WASTE	Water	500 µl	100 µl

CARTRIDGE DATA

Catalog Number: UAC Serial Number: DNA
Length: 24 cm Diameter: 75 mm Coated
Use Count: 0 Active

3.2 dsDNA Analysis Method

Preparation Cycles—The 45 second, high pressure preparation cycle (Prep cycle 1) fills the capillary with fresh run buffer at the beginning of each analysis. The 0 second cycle (Prep cycle 2) dips the capillary and electrode into a vial containing deionized water

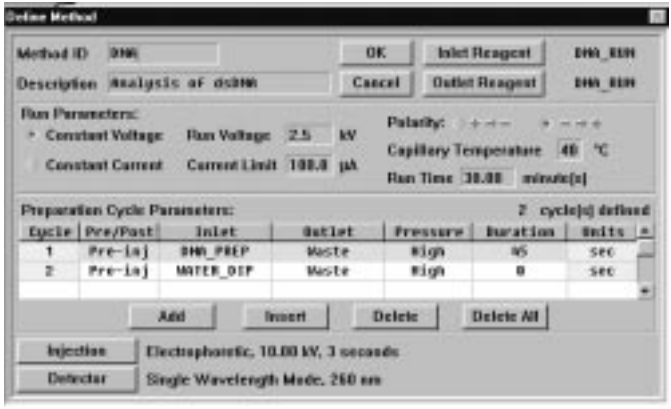
to rinse their surfaces to prevent buffer carryover into the sample vial (see Table 2).

Sample Injection—The best resolution and sensitivity are obtained using electrophoretic injection at constant voltage and a current limit of 100 µA. However, in cases where the sample contains excessive amounts of salt, pressure injection may provide better results. Because of the viscosity of the run buffer, a pressure injection value of 100 psi∗sec or more should be used; this injects a volume of approximately 15 nl.

3.3 Analysis of Multiple Samples

For analysis of multiple samples, a fresh set of run buffer vials should be used every 30 injections.

Table 2. dsDNA Analysis Method for 24 cm Capillary



Section 4
System Shutdown Method

Drying of the buffer at the ends of the capillary may cause the capillary to become plugged. For short term storage, leave the capillary filled with the CE dsDNA Run Buffer and place the capillary ends in test tubes filled with deionized water at 4 °C. For long term storage the capillary can be flushed thoroughly with water and then purge with nitrogen. This flushing may be programmed to run automatically at the end of an automation sequence using the method parameters shown in Table 3. The Shutdown Method

included in the BioFocus software can be used as a template to quickly program the dsDNA Shutdown Method.

Table 3. dsDNA Shutdown Method

ID: END	Description: terminates autosequence			
Prep 1: Pre-Inject from [WATER	] to [Waste	]	180 sec	
Prep 2: Pre-Inject from [NITROGEN	] to [Waste	]	300 sec	
No Injection				
Polarity:	Negative -> Positive			
Run Voltage:	0.00 kV			
Current Limit:	0.30 mA			
Inlet buffer:	[WATER]			
Outlet buffer:	[WATER]			
Cartridge Temperature:	20 degrees Celsius			
Run Time:	1.00 min			
Detector:	Single wavelength 200 nm			
	Channel: 1			
	Range: 0.0200			
	Rise Time (sec): 1.0			
	Lamp(s) turned off at the end of the run group			

Section 5
Optimization of Nucleic Acid Separations

5.1 Desalting

The electrophoretic sample loading method is very sensitive to sample ionic strength. For efficient sample loading, the salt concentration of the sample should be no more than 30 mM. Since the standard reaction buffer for the polymerase chain reaction contains 10 mM Tris + 50 mM KCl, a desalting step before analysis is recommended. Bio-Spin® 6 chromatography columns (catalog number 732-6002) can be used for rapid gel filtration desalting of nucleic acids using the following procedure.

1. Resuspend gel by inverting column five times.
2. Remove cap and tip and allow liquid to drain from bed.
3. Place the column in a collection tube and centrifuge for 2 minutes in a microfuge at 1,000 x g to remove remaining buffer. Discard centrifugate.
4. To exchange buffer, apply 300 µl of the new buffer and centrifuge for 2 minutes in a microfuge at 1,000 x g. Discard centrifugate. Repeat 4 times.
5. Attach a new collection tube to the Bio-Spin column.

6. Carefully pipette the sample (50-100 µl) directly to the center of the column.

7. Centrifuge for 4 minutes at 1,000 x g and retain centrifugate for analysis.

5.2 Preconcentration

Polymerase chain reactions which have gone through many amplification cycles usually have sufficient product DNA to be easily detected without sample preconcentration. If not, ethanol precipitation and reconstitution of the sample in a small volume may be required, using the procedure outlined below.

This procedure is recommended since the final aqueous ethanol wash will desalt the sample. Other concentration methods such as lyophilization may result in high salt levels upon sample reconstitution, which interferes with electrophoretic sample loading (see above).

1. Add 1/10 volume of 3 M sodium acetate (pH 5.2) to the sample.

2. Add 2 volumes cold 95% ethanol.

3. Hold in dry ice + ethanol bath for 2 hours.

4. Centrifuge in microcentrifuge for 15 minutes.

5. Draw off supernatant and add 250 µl 70% aqueous ethanol to the precipitate.

6. Centrifuge for 4 minutes.
7. Draw off supernatant and speedvac for 5–10 minutes to dry.
8. Reconstitute sample in the desired volume of water or buffer.

5.3 Decreasing Time of Analysis

The time of analysis can be shortened by increasing the run voltage, although this can result in some loss in performance. For example, running at 4.0 kV on a 24 cm capillary can shorten the analysis time of the 100 Base Pair Ladder to about 12 minutes.

5.4 Increasing Resolution

Resolution can be increased by increasing the capillary length, however, analysis times will be longer. To maintain the same field strength, the operating voltage can be increased (*e.g.* to 5 kV for a 44 cm capillary).

Resolution also depends upon the width of the sample zone loaded into the capillary; narrow zones improve resolution. Narrow sample zones can be obtained by reducing the injection time and/or the injection voltage for electrophoretic injection, or the psi∗second value for pressure injection.

5.5 Increasing Sensitivity

The amplitude of the detector signal is dependent on the concentration and amount of sample introduced into the capillary dur-

ing sample injection. The amount injected can be increased by increasing the injection voltage and time, or increasing the psi∗second value. Practical limits for electrophoretic injection are approximately 10 kV and 15 seconds, above which peak height is not significantly increased. For pressure injection, limits are dependent on the viscosity of the sample solution and running buffer. Remember that wider sample zones will also reduce resolution.

Higher sensitivity can also be achieved by reducing the sample salt concentration, which produces narrow, concentrated sample zones.

Section 6
Regenerating a Plugged Capillary

If the capillary becomes plugged (as evidenced by zero current, or failure of the liquid to appear at the capillary outlet during manual purging), there are two ways to unplug the capillary.

1. **On-line method**—Leave the capillary cartridge installed in the BioFocus system. Place vials containing 100 µl deionized water in the inlet and outlet carousel. Select Pressure Diagnostics from the toolbar. In the Pressure Diagnostics window, select High Pressure Mode, select the carousel positions containing the water vials, enter 180 seconds for the Maximum Limit for Testing, then press Start. After the 180 second purge period, exit from Pressure Diagnostics and visually check the liquid level in the inlet water vial. If the vial is empty, the capillary has been unplugged.

2. **Off-line method**—Remove the capillary cartridge from the instrument and immerse the capillary ends in hot (70 °C, not boiling) deionized water for 10–15 minutes and check by manually purging with water. Alternatively, immerse the capillary ends in a sonic bath filled with deionized water and sonicate for about 5–10 minutes before purging with water.

Section 7
Product Information

Catalog Number	Product Description
148-4132	**CE dsDNA 4000 Analysis Kit**
148-5041	**CE dsDNA Run Buffer**, contains sieving polymer in 178 mM tris, 178 mM boric acid, 4 mM EDTA, 60 ml
148-5042	**CE dsDNA Diluent Buffer**, 178 mM tris, 178 mM boric acid, 4 mM EDTA, 60 ml
148-2019	**CE dsDNA 100 bp Ladder**, 100 µl at 100 µg/ml in TE buffer, pH 8.0
148-3084	**BioCAP DNA Analysis Capillary**, AAEE coated, 50 cm x 75 µm I.D. x 375 µm O.D.
148-3052	**BioFocus User Assembled Cartridge**

* PCR is covered by U.S. patents owned by F. Hoffmann-La Roche & Co.

** Dynamic sieving CE is covered by U.S. patent 5,089,111 issued to Bio-Rad Laboratories

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