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Life Science Group

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CE dsDNA 1000 Fluorescent Detection and Analysis Kit

Instruction Manual

Catalog Number 148-4133

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For Technical Service Call Your Local Bio-Rad Office
or in the U.S. Call **1-800-4BIORAD** (1-800-424-6723)

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Section 1
Introduction

The CE dsDNA 1000 Fluorescent Detection and Analysis Kit is optimized for use with the BioFocus® capillary electrophoresis laser induced fluorescence LIF[2] detection system and resolves double-stranded (ds) DNA fragments ranging in size from 100 to 1,000 base pairs. The kit is useful for a variety of applications including determination of PCR* yields, 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.

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 BioCap™ capillaries which are coated internally with a novel polymer (polyAAEE), insuring high resolution separations over many runs.

SYBR® Green I, the fluorescent dye included in the kit, is an intercalating monomeric dye with the excitation maximum close to the 488 nm line of the argon ion laser, extremely low intrinsic fluorescence, and very high affinity for dsDNA.

Section 2
CE dsDNA 1000 Fluorescent Detection and Kit Components

The kit contains all the reagents for 80 setups, with 30 runs per setup using the BioFocus LIF[2] detection system.

- Two BioCAP DNA analysis capillaries, 50 cm x 75 µm ID x 375 µm OD
- CE dsDNA run buffer in 2x TBE, 60 ml (2 bottles)
- CE-LIF dsDNA 100 bp ladder, 200 µl at 1.0 µg/ml in TE buffer, pH 8.0
- CE Grade SYBR Green I, 200 µl (Since this fluorescent dye is light sensitive, do not leave exposed to light when not in use. Store the fluorescent dye at 4 °C or less.)
- Instruction manual

2.1 Capillary Preparation and Use

The BioCAP DNA analysis capillary supplied with this kit is designed for use with the BioFocus LIF user assembled capillary cartridge. For information on capillary installation, refer to the instructions included with the BioFocus LIF cartridge assembly kit, catalog number 148-3054. For use with this kit, the capillary cartridge can be assembled with 24 to 44 cm (total length) of capillary installed.

2.2 Using the CE dsDNA 100 Base Pair Ladder

The CE-LIF dsDNA 100 bp 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 (see Figure 1). Pipette 20–50 µl of the CE-LIF dsDNA 100 bp ladder into a 500 µl sample vial. Routinely, the CE-LIF 100 bp ladder should be stored at 4 °C. However, longer term storage at -20 °C may increase shelf life.

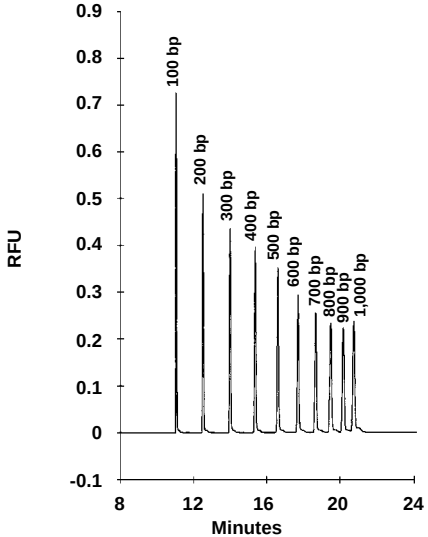


Fig. 1. Separation of 100 bp ladder.

2.3 Sample Preparation

In Figure 1, the concentration of the DNA sample is 1.0 µg/ml. In general, DNA component concentrations should be in the range of 5 to 1,000 pg/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 should be desalted by dialysis or ultrafiltration.

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

The CE dsDNA run buffer should be brought to room temperature before use. It is recommended to filter 12.0 ml of the run buffer using a 1 µm filter (Prep-Disc® filters, catalog number 343-0004). To prepare the CE-LIF run buffer, add 10.0 µl of the CE Grade SYBR Green I to 10.0 ml of the filtered run buffer (**do not filter run buffer after the addition of the fluorescent dye**). The run buffer containing SYBR Green I should be stored in the dark to prevent degradation of the dye. If proper handling is exercised, the run buffer with SYBR Green I should be stable for at least 72 hours. All run buffer vials should be filled to capacity to insure proper contact with the capillary and electrode. Degassing by centrifugation is strongly recommended. Pipette the buffer into 500 µl vials and centrifuge for at least 2 minutes in a microcentrifuge before inserting them into the BioFocus carousels.

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 for up to 30 analyses 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
4	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 (100 µl)	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

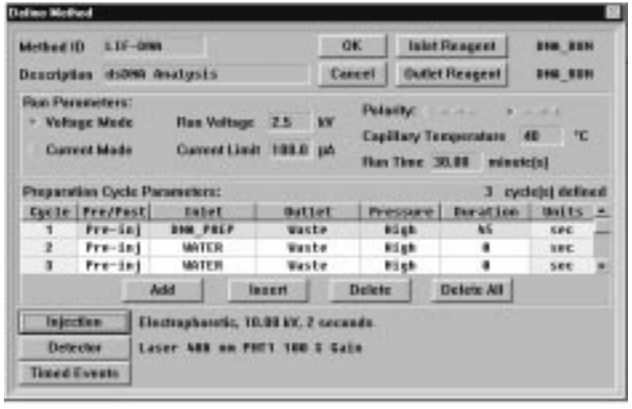
Preparation Cycles—The 45 second, high pressure preparation cycle (Prep cycle 1) fills the capillary with fresh run buffer at the beginning of each analysis. The 0 second cycles (Prep cycle 2 and 3) dip the capillary and electrode into vials containing deionized water to rinse their surfaces and prevent buffer carry-over into the sample vial (see Table 2).

Sample Injection—The best resolution and sensitivity are normally 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



Set the carousel temperature to 20 °C

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 (24 hours), 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 should be flushed thoroughly with water and then purged with nitrogen. This 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:	LIF			
	Channel: 1			
	Laser(s) turned off at the end of the run group			

Section 5
Optimization of Nucleic Acid Separations

5.1 Shortening Analysis Time

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 CE-LIF dsDNA 100 bp ladder to about 12 minutes.

5.2 Increasing Resolution

Resolution can be improved by increasing the length of the capillary, but this will also result in longer analysis times. To maintain the same field strength, the operating voltage should be increased proportionately to the increase in capillary length.

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.

5.3 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.

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 3 minute 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.

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 minute before purging with water.

Section 6
Product Information

Catalog Number	Product Description
148-4133	**CE dsDNA 1000 Fluorescent Detection and Analysis Kit**
148-5041	**CE dsDNA Run Buffer**
148-2021	**CE-LIF ds DNA 100 bp ladder**
148-5100	**CE Grade SYBR Green I Fluorescent Dye**
148-3084	**BioCAP DNA Analysis Capillary**, 2
148-3054	**BioFocus LIF 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.

SYBR Green I is a registered trademark of Molecular Probes, Inc., (M.P.I.) and SYBR Green I is licensed from M.P.I. for CE research.