

DNA Size Standard Calibration Kit

For PCR Product Analysis Using Capillary Electrophoresis
Stock No. DNA-CE

INTRODUCTION

This kit is designed for the separation of low molecular weight dsDNA using a non-gel sieving mechanism. Applications include analyzing the size and purity of PCR products, analyzing restriction digests and calibrating the separation conditions on the Capillary Electrophoresis (CE) work station.

Items Provided

<u>Item</u>	<u>Product No.</u>	<u>Quantity</u>
DNA Separation Medium for CE	D 6799	50 ml
Wash Solution for CE	W 2629	100 ml
Orange G Solution- Internal Standard for CE	O 9007	5 ml
Ethidium Bromide Solution for CE	E 2018	1 ml

Item Needed but not included in the kit: DNA Size Standard for CE (Sigma Product No. D 3421)

A. Protocol for Preparing DNA Size Standards and Unknown DNA Samples for Capillary Electrophoresis

1. DNA Size Standards (Rsa I digest of Phi X 174; 89-1560 bp; Sigma Product No. D 3421)
 - a. Each vial contains 25 µg of DNA in 100 µl of 0.5X TE buffer (5mM Tris-HCl, pH 8.0, 0.5mM EDTA). It is recommended that DNA standard should be divided into aliquots and stored at -20°C or below until ready to use.
 - b. Add 1 µl of Orange G (Sigma Product No. O 9007) to 50 µl of DNA standard prior to run.
2. Unknown DNA samples
 - a. DNA samples, especially PCR products, usually exist in a high salt buffer which can interfere with the electrokinetic injection and the separation. A desalting step before analysis may be necessary. Also if the sample is too dilute, concentration of the sample may be necessary.
 1. For desalting
 - a. Spin column (commercially available)
 - b. Membrane filter dialysis
 2. For preconcentration and desalting
 - a. Ethanol precipitation
 3. For concentration, desalting, and removal of low molecular weight (< 30,000) primers
 - a. Ultrafiltration
 - b. Add 1 µl of Orange G (Sigma Product No. O 9007) to 50 µl of the unknown DNA sample (approx. 4 µg per peak) prior to run.
3. DNA Separation Medium (Sigma Product No. D 6799) and Washing Solution (Sigma Product No. W 2629)
 - a. DNA Separation Medium and Washing Solution should be degassed before use.
 - b. Ethidium Bromide (Sigma Product No. E 2018) may be added to the DNA Separation Medium in 1:100 ratio (v/v) to help the separation of some DNA molecules.

B. Running Conditions

1. Electrophoretic load: 2.0 kV for 20 seconds. Pressure load: 5 psi for 50 seconds.
2. Polarity: Negative to positive
3. Temperature: 25°C
4. Detection Wavelength: 260 nm at 0.02-0.05 AUFS or following manufacturer's instructions.
5. Run time: 30 minutes at 6.5 kV constant voltage.
6. Type of capillary: 50 μm x 36 cm capillary [CElect-H50 from SUPELCO (Sigma Product No. 7-5004) or $\mu\text{Sil DB 17}$ from J & W Scientific].
7. Purge Cycle

To prepare capillary prior to starting electrophoresis,

2 minutes with deionized

3 minutes with DNA Size Separation Medium (Sigma Product Bo. D 6799)

Before each sample application run,

30 seconds with Washing Solution (Sigma Product No. W 2629)

80 seconds with deionized water

180 seconds with DNA Size Separation Medium (Sigma Product No. D 6799)

After completion of electrophoresis,

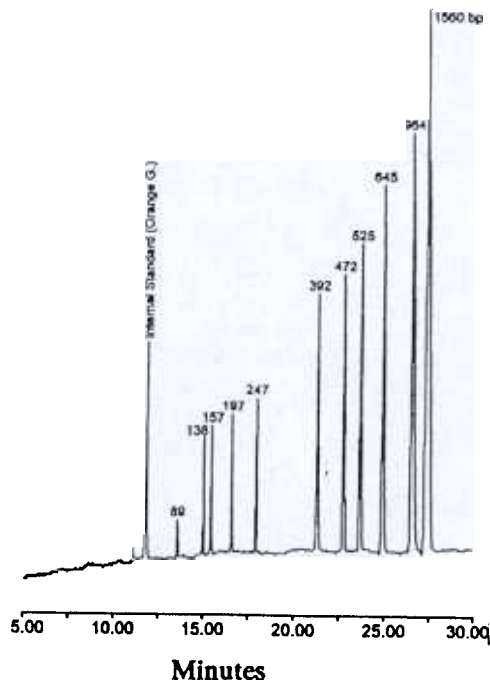
1 minute with deionized water

1 minute with Washing Solution (Sigma Product No. W 2629)

2 minutes with deionized water

3 minutes with dry nitrogen gas

Figure 1. A Typical Electropherogram of DNA Size Standard (Sigma Product No. D 3421) using BioRad BioFocus 3000



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