SIGMA®
ELECTROPHORESIS

1/95

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

Item	Product No.	Quantity
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 µSil 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