CE Dye Kits

(version A)

Catalog Nos. 15101 (CE Dye 503) and 15102 (CE Dye 540)

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Introduction

The CE Dye Kits provide a convenient alternative for the fluorescent labeling of protein samples prior to capillary electrophoresis. Capillary electrophoresis (CE) has proven to be a useful tool in modern cell biology applications, and enables the rapid and high-resolution separation of proteins in a microsample format. These properties have made CE especially useful when performing single-cell protein analyses. However, traditional CE performed using UV absorbance, can suffer from a lack of sensitivity due to the short path length across the capillary.

Derivatization of proteins with fluorescent labels, such as *o*-phthaldialdehyde (OPA), naphthalene-2,3-dicarboxaldehyde (NDA) and 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ), prior to CE separation is one approach that has been used to overcome the sensitivity issues associated with detection using natural protein fluorescence and UV absorption. The most commonly used chemistries utilize the primary amines of lysine residues for attachment of the fluorescent label. This is in part because of the relatively high abundance of lysine residues within proteins, but also because lysine residues tend to be located near the surface of the protein and therefore are accessible for dye-labeling. However, the labeling of protein samples via derivatization can be problematic, due to altered ionic character, which can result in signal masking.

The ionic character of sample proteins is affected when the positive charge of the reactive amine is lost and converted to a neutral charge via the dye:protein conjugation, and because CE utilizes both protein size and charge for separation this is particularly problematic. The effect on ionic character is compounded because most proteins will bind varying amounts of dye label (due to differing lysine content and variable labeling), which results in band broadening and signal masking. Signal masking can also occur when dyes that do not possess large stoke shifts, or display increased quantum yield upon conjugation are used. This is because in CE the free dye label tends to be in huge excess when compared to bound label. In addition, the derivatization chemistry often requires the addition of highly toxic co-factors, such as potassium cyanide.

The CE Dye Kits are designed to overcome the limitations associated with derivatization-based methods for labeling proteins and provide a highly sensitive and convenient solution for CE. The large stoke shift dyes contained within the CE Dye Kits not only maintain the natural ionic character of labeled proteins but also undergo a shortwave length shift upon protein binding and display a 50-fold increase in quantum yield. The result is sensitive protein detection, reduced background and single protein peak detection.

product	format	catalog no.
CE Dye 503	1 kit	15101
CE Dye 540	1 kit	15102

Kit Performance and Benefits

Like other commonly used dyes, the CE dyes utilize the highly abundant and accessible lysine residues for their attachment chemistry. However, the CE dyes overcome the limitation of other dye systems by maintaining the positive charge of the amine group following dye conjugation. This means that proteins labeled with the CE Dyes will not display band broadening or require adjusted ionic character calculations to be performed.

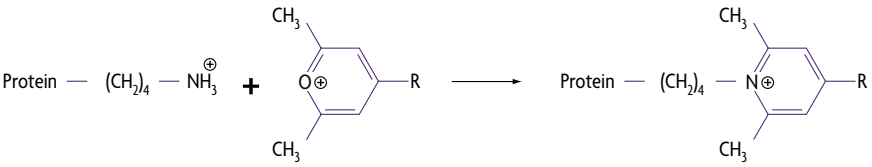

Figure 1: Labeling with CE Dyes maintains each protein's natural charge. Chemical equation depicting the chemistry of CE Dye labeling, which demonstrates the preservation of the positive charge of the lysine residue of a protein. R stands for the respective chromogenic/fluorogenic group.

Kit Components and Storage

Components sufficient for performing 200-300 assays are provided. We recommend storing each component at the temperatures recommended in the table below:

Reagents	Quantity	Storage
CE Dye 503 (Dye Reagent AM1)	0.5 mg	4°C for 6 months
or CE Dye 540 (Dye Reagent AM2)	0.5 mg	4°C for 6 months
Reaction Buffer AM1	25 ml	4°C for 6 months
Reaction Buffer AM2	25 ml	4°C for 6 months

Additional Materials Required

Capillary Electrophoresis device with capacity for laser excitation (approximately 503 or 540 nm) and detection (approximately 600 nm)
Eppendorf tubes
Dimethylsulfoxide (DMSO) or Dimethylformamide (DMF) (for reconstituting the Dye)
Pipettors and tips

Preparation of Reagents

Dye Stock Solution

The CE Dye 503 and CE Dye 540 are supplied lyophilized. Prepare the Dye Stock Solution by resuspending the lyophilized Dye in 635 µl DMSO or DMF, respectively. This stock solution can be stored in the dark at 4°C for 6 months.

Protein labeling using CE Dye 503

1. Remove kit contents from 4°C and bring all components to room temperature before use.
2. Prepare unknown protein samples by adding between 1-20 µl of sample to separate tubes and adding the provided Reaction Buffer AM1 to make a final volume of 46 µl and mix. **NOTE**: Some contaminating substances may interfere with the assay, please refer to the Table on page 4 for more information.
3. Add 2 µl of the CE Dye 503 Stock Solution to each sample tube and mix thoroughly.
4. Incubate samples at room temperature (20-25 °C) for 30 minutes and analyze by CE. **NOTE:** The free label is blue in color and upon reaction with a primary amine is converted to a red product and the signal intensity is stable over a period of 10 minutes to 2 hours.

Protein labeling using CE Dye 540

1. Remove kit contents from 4°C and bring all components to room temperature before use.
2. Prepare unknown protein samples by adding between 1-20 µl of sample to separate tubes and adding the provided Reaction Buffer AM2 to make a final volume of 43 µl and mix. **NOTE**: Some contaminating substances may interfere with the assay, please refer to the Table on page 4 for more information.
3. Add 2 µl of the CE Dye 540 Stock Solution to each sample tube and mix thoroughly.
4. Incubate samples at 50 °C for 1 hour and analyze by CE. **NOTE:** The free label is purple in color and upon reaction with a primary amine is converted to a pink product. The product formed is stable for 3 months.

References

1. Craig D.B., Wetzl B.K., Duerkop A. and Wolfbeis O.S. (2005) *Electrophoresis* 26: 2208-2213.

Appendix

Section A. Troubleshooting

I. Separation and Detection

Typical conditions for CE separation of CE-labeled samples are as follows:

- Mobile phase: 2.5 mM Sodium tetraborate buffer (pH 9.3), plus 1.4 mM (CE Dye 503) or 3.5 mM (CE Dye 540) SDS.
- Operating Voltage: 20 KV
- Excitation: Ar^+ (CE Dye 503) or HeNe laser (CE Dye 540)
- Emission: 600 nm

II. Buffer Compatibility and Contaminating Substances

A number of common contaminants have been tested with the Fluorescent Protein Quantification Kit, and most are well tolerated; however, samples containing high concentrations of free amines are not recommended (See Table below).

Contaminant	Final Concentration in the assay	Result
Sodium Chloride	20 mM	OK
Magnesium Chloride	2 mM	OK
Tris	10 mM	OK
Ammonium Sulfate	5 mM	OK
Tween	0.001%	Not Recommended
Triton	0.001%	Not Recommended
SDS	0.04%	OK

*BSA standards were assayed in the presence or absence of contaminants at the indicated final concentrations.

II. Excitation and Emission Filters

The excitation maxima for the CE Dye 503 and CE Dye 540 bound to protein are 503 and 540 nm, respectively, and share a common emission maximum of 600 nm. In order to maximize the signal from the fluorophore, it is usually best to choose filters that are spectrally separated and slightly offset from the peak emission wavelengths.

Section B. Related Products

TransAM™ Family Kits	Units	Catalog No.
TransAM™ AP-1 Family	2 x 96-well plates	44296
TransAM™ GATA Family	2 x 96-well plates	48296
TransAM™ HNF Family	2 x 96-well plates	46296
TransAM™ IRF Family	2 x 96-well plates	45296
TransAM™ MAPK Family	2 x 96-well plates	47296
TransAM™ Flexi NFκB Family	2 x 96-well plates	43298
TransAM™ NFκB Family	2 x 96-well plates	43296
TransAM™ STAT Family	2 x 96-well plates	42296
Sandwich ELISAs		
FunctionELISA™ IκBα	1 x 96-well plates	48005
	5 x 96-well plates	48505
FunctionELISA™ TRAIL	1 x 96-well plates	48010
	5 x 96-well plates	48510
FunctionELISA™ Cytochrome c	1 x 96-well plates	48006
	5 x 96-well plates	48506
NR Sandwich AR	1 x 96-well plates	49196
	5 x 96-well plates	49696
NR Sandwich ER	1 x 96-well plates	49296
	5 x 96-well plates	49796
NR Sandwich PR	1 x 96-well plates	49396
	5 x 96-well plates	49896
Chromatin Immunoprecipitation		
ChIP-IT™ Kit	25 reactions	53001
ChIP-IT™ w/o controls	25 reactions	53004
ChIP-IT™ Shearing Kit	10 reactions	53002
ChIP-IT Enzymatic	25 reactions	53006
ChIP-IT™ Enzymatic w/o controls	25 reactions	53007
Enzymatic Shearing Kit	10 reactions	53005
Salmon Sperm DNA/ Protein G agarose	25 reactions	53003
Co-Immunoprecipitation		
Nuclear Complex Co-IP Kit	50 reactions	54001
Sample Preparation		
Nuclear Extract Kit	100 reactions	40010
	400 reactions	40410
Mitochondrial Fractionation Kit	100 reactions	40015
GAPDH Whole-cell Normalization Kit	1 x 96-well plate	48007
	5 x 96 well-plates	48507
Fluorescent Detection		
CE Dye 503	1 kit	15101
CE Dye 540	1 kit	15102

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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