



Operating and Instruction Manual

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CElixir-plus™

Kits for non UV absorbing Cations and Aliphatic Amines

Instructions for use.

Background Information on Method

Indirect Detection:

Small, positively charged molecules (cations) such as NH₄, K, Na Mg, Ca and aliphatic amines are not UV absorbing and therefore require an “indirect method of detection” when using UV detectors. The CElixir-plus™ buffers are UV absorbing and will absorb most of the UV. When a non absorbing analyte such as an aliphatic amine passes by the detection window, the detector senses a decrease in absorption and records the “peak”.

Dynamic Coating for Reproducibility:

CElixir-plus™ uses a dynamic coating principle which means for each run, the capillary wall is treated to produce a very precise EOF. If an analyte adsorbs to the wall, it is rinsed clean with LiOH between runs and a fresh coating is reapplied; the same EOF results.

INTRODUCTION TO CELixir-plus™

CELixir-plus™ enhances the analysis of small, positively charged molecules by CE. The CELixir-plus™ kits provide a dynamic coating when applied to the surface of the capillary wall produces a stable and highly reproducible EOF. The coated surface is propagated with negative charges creating a robust, reproducible EOF.

By following the simple instructions contained in this manual, it becomes very easy to separate cations and aliphatic amines.

Coating Definition.

The proprietary properties of the CELixir-plus™ dynamic coating system achieves its uniform EOF characteristics by a stable bond formed between the polycations in the Initiator Solution (A) and the capillary wall.

Run Buffer and Background Electrolyte.

No other buffers or Background Electrolytes (BGE) are needed. The Separating Buffer, included in the kit is the run buffer and the BGE. The Separating Buffer contains chromophores.

Matched Solutions.

Each CELixir-plus™ kit is supplied with 14ml of Initiator Solution (A) and 14ml of Accelerator Solution (B), 14ml of Conditioner Solution and 42ml of Separating Buffer. These solutions are provided with a Serial Number and must be used together as a matched set. Initiator Solutions of one kit cannot be used with the Accelerator Solutions of another kit. It is important that care is taken to use the correct matched set for reproducible results. This kit is designed to provide 200 tests.

Reagents and Materials.

Materials Needed to Separate Aliphatic Amines and Cations

CElixir-plus Initiator	Solution (A) Included
CElixir-plus Accelerator	Solution (B) Included
CElixir-plus Conditioner	Solution, Included
CElixir-plus Separating Buffer	Solution, Included
Capillary	Bare Fused Silica, typically 75µm ID by 60cm long. Not included
CE Grade Water	Not included
Vials	Not Included

Preparation

Sample Preparation.

Depending on the concentration of your analyte, the sample can be injected neat or diluted with CE Grade Water. Best results are obtained when pH of the sample is lower or equal to pH 4.2. Adjustment of the pH may be done by addition of LiOH to bring the pH higher or you can add HCL if want to lower the pH, avoid unnecessary dilution.

Hydrodynamic (Pressure) Injection Technique:

When using this technique, it may be optimal to dissolve your sample in CE Grade Water.

Electrokinetic (Current) Injection Technique:

When using this technique, it may be optimal to dissolve your sample in CE Grade Water. See below for Autosampler Vial filling procedure.

Run Buffer and CElixir-plus™ Solution.

The solutions provided in the CElixir-plus™ kits are ready to use and require no further preparation. CElixir-plus™ kits do not operate correctly with any other run buffer and should not be used.

Operation

Refer to your instrument manual for general operation and instructions on how to perform suitable separations.

Starting with a New Capillary.

When using a new capillary follow the recommended procedures of the manufacturer for cutting this capillary. A true perpendicular cut to the ends of the capillary are vital to the success of any CE separation. For cutting a Simplus capillary please refer to our website at www.mtc-usa.com; enter the Electrophoresis pages to find the Capillary Electrophoresis Primer. The direct URL is <http://www.mtc-usa.com/cutcap.htm>.

It is highly recommended to burn 2mm of the polyimide from each end of the capillary for injection ruggedness and reproducibility.

Capillaries should be dedicated to CElixir™ products.

Initiate the Capillary.

1. Install the new capillary by following the CE instrument manufacturer's instructions.
2. Rinse the newly installed capillary with CElixir-plus™ Conditioning Solution for one (1) minute.
3. Rinse the capillary with CElixir-plus Initiator Solution (A) for (1.0) minute.
4. Rinse the capillary with CElixir-plus Accelerator Solution (B) for (2.0) minutes.
5. Rinse the capillary with CElixir-plus Conditioner Solution for (0.5) minute.
6. Rinse the capillary with CE Grade Water for (0.5) minute.
7. The capillary is ready to be used with the CElixir-plus™ system.

Example of Sample Vials (for Beckman P/ACE MDQ)

Using other CE instruments is easy. Please modify this method according to your instruments requirements.

Always use vials that are recommended by your CE instrument or their exact equivalent.

DO NOT OVER-FILL the VIALS.

Load your CE instrument's autosampler with vials containing CELixir-plus, reagents and samples. **3 x 20 analyses.**

Reagent	Vial	Buffer Inlet	Buffer Outlet
Conditioner Solution	3 Vials	A1..A3	
Initiator (A)	3 Vials	B1..B3	
Accelerator (B)	3 Vials	C1..C3	
CE Grade Water	9 Vials	B4..B6, D4..D6,F4..F6	C4..C6
Separating Buffer	6 Vials	C4..C6. E4..E6	B4..B6
Empty (waste) Vial	3 Vial		A4..A6
Samples	Vials: Buffer Inlet	S1	

Method of Separation.

Follow a method specific for your CE instrument. Following is a sample separation method.

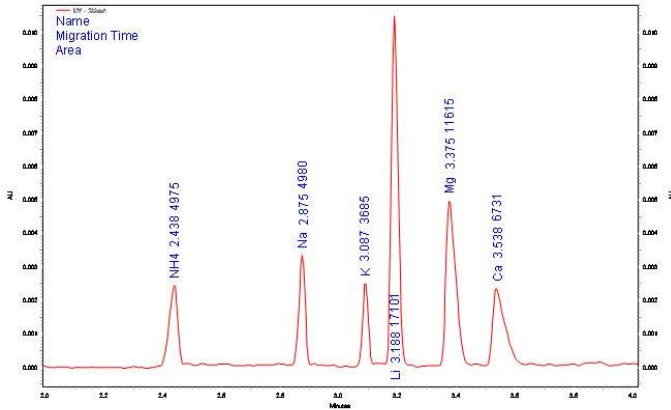
Temperature:	25°C
Detection Wavelength:	200 nm for CELixir-plus™
Detection Mode:	Indirect for CELixir-plus™ For DAD at 200nm use 10nm Bandwidth (data rate 4Hz, filter normal peak width 16-25)
Polarity:	Cathodic (Normal)
Current:	30 kV

Generic Program for Coating/Injection and Separation after Capillary is Initiated. See previous section on New Capillaries.

Time	Function	Value	Duration	Inlet Vial	Outlet Vial	Comments
	Rinse	20.0 psi	0.30 min	Initiator (B1)	Empty (A4)	
	Rinse	20.0 psi	0.50 min	Accelerator(C1)	Empty (A4)	
	Rinse	20.0 psi	1.50 min	Sep Buffer (C4)	Empty (A4)	
	Wait		0.20 min	Water (B4)	Water (C4)	Wait Time
	Injection	0.5 psi **	5.0 sec	Sample (S1)	Sep Buffer (B4)	
	Injection	0.1 psi	10.0 sec	Water (D4)	Sep Buffer (B4)	Water Plug
0.0	Separation	30 kV	5.5 min*	Sep Buffer (E4)	Sep Buffer (B4)	1min Ramp
2.0	Auto Zero					
5.5*	Stop Run					
5.5*	Rinse	20.0 psi	0.50min	Conditioner (A1)	Empty (A4)	
6.00*	Rinse	20.0 psi	0.50min	Water (F4)	Empty (A4)	
6.,5	End					

* Time adapted to individual separation requirements. 1 psi = 0.06895 bar.

** For Beckman P/ACE MDQ, the water plug should occur at 0.1psi for 10 seconds



Sample Electropherogram Above.

Change Buffers every 20 runs or depending on sample matrix. When contamination occurs, change water vials used for rinsing more often.

Lithium Peaks:

Lithium can appear as a system peak. If you are analyzing for Lithium, use Sodium Hydroxide Solution 0.1N as the Conditioner instead of the supplied Conditioner.

Re-use of Capillary.

Do not use the capillary for any other separations other than CELixir-plus™ separations once you have coated it with CELixir-plus™ Initiator Solution.

Between runs rinse the capillary with Conditioner solution with at least one column volume (typically 0.5 minutes) then rinse it with CE Grade Water with the same column volume (typically 0.5 minutes), then start the separation as described in Generic Program listed on page 6.

Storage of the Capillary requires a rinse with CE Grade Water with a volume equivalent to 2 column volumes (2 times the capillary length, typically 1 minute at 20 psi).

CElixir-plus™ Solutions Storage.

DO NOT REFRIGERATE CElixir-plus Solutions.

CElixir-plus™ Solutions should be capped immediately after use and stored at room temperature (18°C to 26°C).

Support.

For technical support or customer service contact:

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Technical Information

Initiator Solution:	14ml pH 4.2, in 4-aminopyridine
Accelerator Solution:	14ml pH 4.2, in 4-aminopyridine
Separating Buffer:	42ml pH 4.3, in Malic Acid(20mM) 4-aminopyridine, 18-crown
Conditioner Solution:	14ml Lithium Hydroxide 0.1mol/L

CElixir-plus, Cat. No. 06300-01 This kit is designed for approximately 200 test runs.

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