

## Reordering Information.

| <u>Catalog No.</u> | <u>Description</u>  |
|--------------------|---|
| 06100.2.5          | Kit, contains 30ml of Initiator Solution and 90ml of Accelerator Solution pH 2.5, Phosphoric Acid Buffer System. Direct Detection.                  |
| 06100-5.4          | Kit, contains 30ml of Initiator Solution and 90ml of Accelerator Solution pH 5.4 (Pyridine-dicarboxylic Acid Buffering System). Indirect Detection. |
| 06100-8.2          | Kit, contains 30ml of Initiator Solution and 90ml of Accelerator Solution pH 8.2 (Pyridine-dicarboxylic Acid Buffering System). Indirect Detection. |



# *Operating and Instruction Manual*

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# CElixirOA™

Kits for Organic Acids and Anions

## Instructions for use.

### **Background Information on Method**

#### **Indirect Detection:**

Small negatively charged molecules (anions) such as aliphatic organic acids are not UV absorbing and therefore require an “indirect method of detection” when using UV detectors. The CElixirOA pH 5.4 and pH 8.2 buffers contain a “chromophore” that will completely absorb all the UV energy and produces a detector response that is full and “off scale”. When a non absorbing analyte such as an organic acid passes the detection window, the detector senses a decrease in absorption and records the “peak”.

#### **Direct Detection:**

Some anions such as Nitrate, Nitrate and bromide as well as aromatic acids and unsaturated aliphatic acids are UV absorbing. They separate best in very acidic conditions such as pH 2.5 and for this reason CElixirOA™ pH 2.5 is available without chromophores and operates in the same way cathodic buffer systems do with regard to detection.

#### **Reverse Polarity:**

Due to the negative charge of the anions, they migrate toward the anode (positive). To have these anions flow in the direction toward the detection window, the polarity of the CE instrument is reversed. All other conditions remain “normal” when using these kits and no other changes are required. Vials are loaded in the same position as before.

#### **Dynamic Coating for Reproducibility:**

CElixirOA™ uses a dynamic coating principle that means for each run, the capillary wall is treated producing a very consistent EOF. If any analyte adsorbs to the wall, it is rinsed clean with NaOH between runs and a fresh coating is reapplied; for the same EOF. This coating will reverse the EOF direction normally produced by bare fused silica capillaries, see above.

The individual vials can be reused until the volume is too low for the system to work or until the buffers are depleted.

DO NOT REFILL The vials...dispose of used vials when reagents and buffers need replenishment.

#### **Re-use of Capillary.**

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

**Between runs** rinse the capillary with 0.1M NaOH 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).

#### **CElixirOA™ Solutions Storage.**

**DO NOT REFRIGERATE CElixirOA Solutions.**

CElixirOA™ Initiator and Accelerator Solutions of all pH ranges 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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Fax: 1-732-578-9777  
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[www.MTC-USA.com](http://www.MTC-USA.com)

## Sample Vials.

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 CELixirOA, reagents and samples.

| Reagent         | Vial Position  |
|-----------------|--|
| NaOH 0.1M       | 1 Vial: Buffer inlet   |
| Initiator (A)   | 1 Vial: Buffer inlet   |
| CE Grade Water  | 2 Vials: Buffer inlet (one for Rinse(1) /one for Water Plug Injection (2))                                   |
| Accelerator (B) | 3 Vials:2 in Buffer inlet (one for Rinse(1)/one for Run Buffer(2))<br>1 in Buffer outlet(3) (For Separation) |
| Empty Vial      | 1 Vial: Buffer Outlet  |
| Samples         | Vials: Buffer Inlet  |

## 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 CELixirOA™ pH 2.5<br>233 nm for CELixirOA™ pH 5.4 and pH 8.2   |
| Detection Mode:       | Direct for CELixirOA™ pH 2.5<br>Indirect for CELixirOA™ pH 5.4 and pH 8.2 |
| Polarity:             | Anodic (Inverse)  |
| Current:              | 30 kV for CELixirOA™ pH 5.4 and pH 8.2<br>20 kV for CELixirOA™ pH 2.5     |

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    | Summary    |
|-------|------------|------------|----------|----------------|----------------|------------|
|       | Rinse      | 20.0 psi   | 0.20 min | Initiator      | Empty          | Fwd High   |
|       | Rinse      | 20.0 psi   | 0.50 min | Accelerator(1) | Empty          | Fwd High   |
|       | Injection  | 0.5 psi    | 5.0 sec  | Sample         | Accelerator(3) | Sample     |
|       | Injection  | 0.5 psi ** | 1.0 sec  | Water(1)       | Empty          | Water Plug |
| 0.0   | Separation | 30 kV      | 7.0 min* | Accelerator(2) | Accelerator(3) | 1min Ramp  |
| 2.0   | Auto Zero  |            |          |                |                |            |
| 7.0*  | Stop Run   |            |          |                |                |            |
| 7.0*  | Rinse      | 20.0 psi   | 0.50min  | NaOH           | Empty          | Forward    |
| 7.50* | Rinse      | 20.0 psi   | 0.50min  | Water(2)       | Empty          | Forward    |

\* 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

## INTRODUCTION TO CELixirOA™

CELixirOA™ enhances the analysis of small, negatively charged molecules by CZE. The CELixirOA™ kits provide a dynamic coating when applied to the surface of the capillary wall producing a stable and highly reproducible EOF. The coated surface is highly propagated with positive charges creating a reverse EOF.

By following the simple instructions contained in this manual, it becomes very easy to separate anions and aliphatic organic acids.

### Coating Definition.

The proprietary properties of the CELixirOA™ 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. This coating covers the wall with a greatly increased number of positive charges thus increasing the speed of the EOF.

### Run Buffer and Background Electrolyte.

No other buffers or Background Electrolytes (BGE) are needed. The Accelerator (B) solution of the kit is the run buffer and the BGE. The Accelerator Solution contains the chromophores when required and does not contain them in the pH 2.5 kit for direct detection.

### Matched Solutions.

Each CELixirOA™ kit is supplied with 30ml of Initiator Solution (A) and 90ml of Accelerator Solution (B). 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.

## Reagents and Materials.

### Materials Needed to Separate Organic Acids and Anions

|                       |   |
|-----------------------|---|
| CElixirOA Initiator   | Solution (A) in 1 Vial for Coating                                |
| CElixirOA Accelerator | Solution (B) in 3 Vials, one for Injection,<br>Two for Separation |
| Capillary             | Bare Fused Silica, typically 75µm ID by 60cm long.                |
| CE Grade Water        | in 2 Vials, one for rinse and one for water plug                  |
| NaOH Solution         | 0.1M in 1 Vial for Initiating the Capillary                       |
| Waste                 | Empty Vial  |

### Included in the CElixirOA™ kits:

Initiator and Accelerator Solutions of matched pH and detection method are included as individual kits. See section on Ordering Information. *Capillaries, CE Grade Water, NaOH solution and Vials are NOT included in the kits.*

## 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 higher or equal to the pK's of the analytes. Adjustment of the pH may be done by addition of NaOH to bring pH to 5 plus or minus 0.1, avoid unnecessary dilution.

If your sample contains pure acids, you must bring the pH of your sample up to pH 5 plus or minus 0.1. An example is Vinegar or when searching for impurities in a solution of organic acids.

#### *Hydrodynamic (Pressure) Injection Technique:*

When using this technique, it may be optimal to dissolve your sample in 0.005M NaOH.

#### *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 CElixirOA™ Solution Preparation.

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

### Pre-Adjusting the pH of the CElixirOA™ Accelerator (B) for optimal separations.

If better selectivity is desired or required of some analytes, the pH of CElixirOA pH 5.4 and CElixirOA pH 8.2 can be adjusted by the careful addition of the accelerator solutions together. It is not recommended to adjust the pH of the CElixirOA™ pH 2.5 Accelerator Solution.

## 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 MicroSolvCE capillary please refer to our website at [www.MTC-USA.com](http://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 CElixirOA™ but the same capillary can be used by any of the three kits (different pH's) offered.*

Initiate the Capillary.

1. Install the new capillary by following the CE instrument manufacturer's instructions.
2. Rinse the newly installed capillary with NaOH 0.1M for five (5) minutes.
3. Rinse the capillary with CE Grade Water for one (1) minute.
4. The capillary is ready to be used with the CElixirOA™ system.