TECHNICAL MANUAL

OncoMate™ 5C Matrix Standard

Instructions for Use of Product
MD3850

INSTRUCTIONS FOR
USE OF PRODUCT
MD3850

IVD

Rev 0
TM597

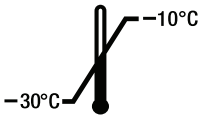
PROMEGA
2800 Woods Hollow Rd.
Madison, WI USA

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

OncoMate™ 5C Matrix Standard

Promega

Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Lot number
	Store at –30°C to –10°C.		Manufacturer
	Do not reuse		Irritant
	Catalog number		Contains sufficient for <n> tests
	Use by		Protect from light
	Consult instructions for use		Authorized Representative
	Conformité Européenne		

1. Product Name

OncoMate™ 5C Matrix Standard

Part No. MD3850

2. Intended Use

The OncoMate™ 5C Matrix Standard is intended for in vitro diagnostic use as an IVD medical device accessory used with the OncoMate™ MSI Dx Analysis System (Cat.# MD3140). The standard is used for spectral calibration of capillary electrophoresis instruments prior to analysis of amplification products generated with the OncoMate™ MSI Dx Analysis System. This product is intended for professional use only.

3. Summary and Explanation

The OncoMate™ MSI Dx Analysis System employs multiplex polymerase chain reaction (PCR) to generate DNA fragments labeled with three different fluorescent dyes: fluorescein, JOE and TMR-ET. During analysis by capillary electrophoresis, the dye-labeled DNA fragments are separated and detected by the instrument alongside the WEN-dye-labeled Size Standard 500. Prior to analysis, the capillary electrophoresis instrument must be calibrated with the OncoMate™ 5C Matrix Standard to distinguish fluorescent signals from the specific dyes used in the assay. The OncoMate™ 5C Matrix Standard[a,b] consists of DNA fragments labeled with five different fluorescent dyes (fluorescein, JOE, TMR-ET, CXR-ET and WEN) in one tube. The spectral calibration is performed according to the instrument manufacturer's instructions.

Follow the instrument manufacturer's user manual for the appropriate maintenance procedures for your instrument. For example, a new spectral calibration must be run after performing major maintenance on the capillary electrophoresis system, such as changing the excitation source (e.g., laser), calibrating or replacing the CCD camera, or changing the polymer type or capillary array. A new spectral calibration also should be performed if bleedthrough peaks that interfere with data analysis are observed.

4. Test Principle

During the data collection portion of a capillary electrophoresis instrument run, DNA fragments labeled with fluorescent dyes are exposed to a light source and emit light of different wavelengths. These emissions are captured by an integrated camera for further analysis. Multiple fluorescent dyes are used to allow simultaneous detection of similarly sized DNA fragments.

Each fluorescent dye used by the OncoMate™ MSI Dx Analysis System has maximum light emission at a unique wavelength but emits light over a range of wavelengths. Where the spectral emissions from these dyes overlap, identifying the dye source of the emission is confounded, interfering with data analysis. Therefore, to analyze microsatellite data resulting from the use of multiple fluorescent dyes, the instrument analysis software must distinguish dye emission spectra.

4. Test Principle (continued)

A spectral calibration standard, or matrix standard, consists of fluorescently labeled DNA fragments that are analyzed during a spectral calibration. The capillary electrophoresis data collection software analyzes the emission spectra of these dye-labeled fragments to characterize spectral overlap and create a multicomponent deconvolution matrix that is specific to each capillary of the calibrated array. The deconvolution matrix is applied automatically to raw sample data in subsequent analysis runs to isolate and attribute observed fluorescence to individual dye sources.

5. Product Components and Storage Conditions

5.1 Materials Provided

This product contains sufficient reagents to perform five spectral calibrations. The following materials are included:

COMPONENT	SIZE	PART#
5C Matrix Mix	**150µl**	**MD430A**

Includes: Fluorescently labeled DNA fragments

Storage Conditions: Post-amplification area; −30°C to −10°C prior to use; 2°C to 10°C following first use. Protect from light.

COMPONENT	SIZE	PART#
Matrix Dilution Buffer	**5 × 200µl**	**MD191A**

Includes: Tris-EDTA-based buffer

Storage Conditions: Post-amplification area; −30°C to −10°C prior to use; 2°C to 10°C following first use.

5.2 Storage and Handling

Store the OncoMate™ 5C Matrix Standard with post-amplification reagents. Upon receipt, store all components at −30°C to −10°C in a nonfrost-free freezer, protected from light. Do not store reagents in the freezer door, where the temperature can fluctuate. After the first use, store the OncoMate™ 5C Matrix Standard components at 2–10°C, protected from light, for up to 3 months. The OncoMate™ 5C Matrix Standard is light-sensitive; dilute the 5C Matrix Mix in Matrix Dilution Buffer in the provided amber tube. Store the diluted 5C Matrix Mix at 2–10°C for up to 6 days.

Note: Do not refreeze the OncoMate™ 5C Matrix Standard components.

5.3 Materials Not Provided

Laboratory Reagents

- Hi-Di™ Formamide (e.g., Applied Biosystems Cat.# 4404307)

The use of Hi-Di™ Formamide is required for spectral calibration using the OncoMate™ 5C Matrix Standard. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take appropriate precautions when handling this substance. Wear gloves, protective clothing and safety glasses when executing the protocols below.

Failure to follow the recommended protocols for storing spectral calibration reagents, performing the spectral calibration, or accepting or rejecting the results of the spectral calibration may result in bleedthrough ("pull-up") artifact peaks during capillary electrophoresis analysis of OncoMate™ MSI Dx Analysis System amplification products. Bleedthrough peaks may obscure assay results or complicate data interpretation.

Laboratory Equipment

- set of calibrated precision pipettes capable of delivering 1µl to 1,000µl
- aerosol-resistant pipette tips (10µl to 1,000µl)
- 1.5ml microcentrifuge tubes
- centrifuge compatible with 96-well plates (e.g., "mini plate centrifuge")
- microcentrifuge tube racks
- vortex mixer
- nonfrost-free freezer at –30°C to –10°C
- refrigerator at 2°C to 10°C

Instruments and Accessories

- Capillary electrophoresis instrument and associated supplies, including polymer, array, buffers, water and maintenance items recommended by the instrument manufacturer.
- MicroAmp® Optical 96-Well Reaction Plate (e.g., ThermoFisher Cat.# 4306737)

6. Capillary Electrophoresis Instrument Requirements

OncoMate™ MSI Dx Analysis System amplification products are separated and analyzed by capillary electrophoresis after spectral calibration with the OncoMate™ 5C Matrix Standard. Performance of the OncoMate™ 5C Matrix Standard and OncoMate™ MSI Dx Analysis System was evaluated using the Applied Biosystems® 3500 Dx Genetic Analyzer run with Fragment Analysis settings and configured with POP-7® Polymer and a 50cm capillary array. Instruments compatible with the OncoMate™ MSI Dx Analysis System and OncoMate™ 5C Matrix Standard will share the following specifications:

Number of dyes detected: ≥5

Capillary array length: array lengths suitable for single-base resolution, including 50cm

Separation matrix: POP-7® polymer or equivalent

Excitation wavelength (approximate): 480nm to 520nm

Detection optics: Promega dyes require emission capture from approximately 500nm to 630nm

Resolution range: 1bp resolution from 60bp to ≥300bp

Sizing precision (Repeatability, expressed as standard deviation): ≤0.15bp across a range of 60bp to ≥300bp

7. Preparation of the Capillary Electrophoresis Instrument

Follow the manufacturer's instructions for operation and maintenance of the selected capillary electrophoresis instrument. Spectral calibration must be performed using the same capillary electrophoresis polymer type and the same array used for the subsequent analysis of OncoMate™ MSI Dx Analysis System amplification products.

1. Ensure that instrument polymer and buffers are not expired and that a sufficient number of samples or injections are available to complete the calibration.
2. If applicable, preheat the capillary electrophoresis oven according to the manufacturer's instructions for at least 30 minutes before starting a run.
3. A spatial calibration of the instrument may be required prior to performing spectral calibration. See the user guide for the selected instrument for additional information. If the capillary electrophoresis instrument requires spatial calibration and you have not performed one yet, do so now.

   Note: We recommend the use of a new capillary array, fresh polymer and fresh buffer for best results.

8. Analysis of the Matrix Standard

1. At first use, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2–10°C, protected from light.
2. Vortex the 5C Matrix Mix for 10–15 seconds at maximum speed. Add 10µl of 5C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds at maximum speed. Record dilution date on the tube.

   Note: The diluted 5C Matrix Mix can be stored at 2–10°C for up to 6 days.

3. Add 10µl of the diluted 5C Matrix Mix (prepared in Step 2) to 500µl of Hi-Di™ Formamide. Vortex for 10–15 seconds at maximum speed.

4. Add 15µl of formamide-matrix mix (prepared in Step 3) to each well that the capillary array will sample during spectral calibration. The number of wells required for spectral calibration depends on the specific instrument and array selected for downstream analysis of OncoMate™ MSI Dx Analysis System amplification products.

5. Cover the plate according to the instrument manufacturer's instructions, and briefly centrifuge the plate to bring the mixture to the bottom of each well and to remove air bubbles.

Note: Do not heat-denature the 96-well plate containing the formamide-matrix mixture. Discard any unused formamide-matrix mixture.

6. Load the plate onto the selected capillary electrophoresis instrument and perform the spectral calibration according to instructions provided in the instrument user guide. Table 1 provides instrument settings used for spectral calibration with the OncoMate™ 5C Matrix Standard. Some settings described in Table 1 may not be applicable for all capillary electrophoresis instrument platforms. Consult the capillary electrophoresis instrument user guide or contact your local Promega Branch Office or Distributor or email: **genetic@promega.com** for additional information.

   Note: You will need to create a new Dye Set before completing your first spectral calibration with the OncoMate™ 5C Matrix Standard. Use either a Dye Set that is specific for the dyes used in the matrix standard (fluorescein, JOE, TMR-ET, CXR-ET and WEN) or a generic dye set (e.g., "AnyDye" or equivalent) as the template. The use of an inappropriate Dye Set for spectral calibration may result in diminished dye-signal balance among the fragments detected during fragment analysis.

Table 1. Settings Used for Spectral Calibration with the OncoMate™ 5C Matrix Standard.

Category	Settings
Number of dyes (i.e., colors)	5
Dye Set	Dye-specific[1], AnyDye or equivalent
Dye Order	1, orange; 2, red; 3, yellow; 4, green; 5, blue
Minimum Quality Value	0.95
Maximum Condition Number	8.0
Sensitivity	0.4
Locate Start Point After Scan	300
Locate Start Point Before Scan	5000
Limit Scans to	6500

[1]Dye set specifically designed for fluorescein, JOE, TMR-ET, CXR-ET and WEN

9. Interpretation of Results

1. Upon completion of the spectral calibration run, review the spectral calibration results. For each capillary, check the Quality Value and Condition Number of the spectral calibration and inspect the spectral emission data displayed. Passing capillaries will have a Quality Value of ≥0.95 and a Condition Number of ≤8.0. Ensure that the order (left to right) of the resolved fragment peaks in the intensity vs scan number display is orange, red, yellow, green and blue (Figure 1, Panel A). Ensure that the order (left to right) of the dye signals in the emission spectra display is blue, green, yellow, red and orange (Figure 1, Panel B).

   Note: The Quality Value for each capillary for a passing spectral calibration is typically ≥0.98.

2. If all capillaries passed and if the corresponding emission data were displayed correctly, accept the spectral calibration. Otherwise, reject the spectral calibration, and refer to Section 10, Troubleshooting.

   Note: If a "borrowing" option was used during spectral calibration, see the capillary electrophoresis instrument user guide for the required number of passing capillaries.

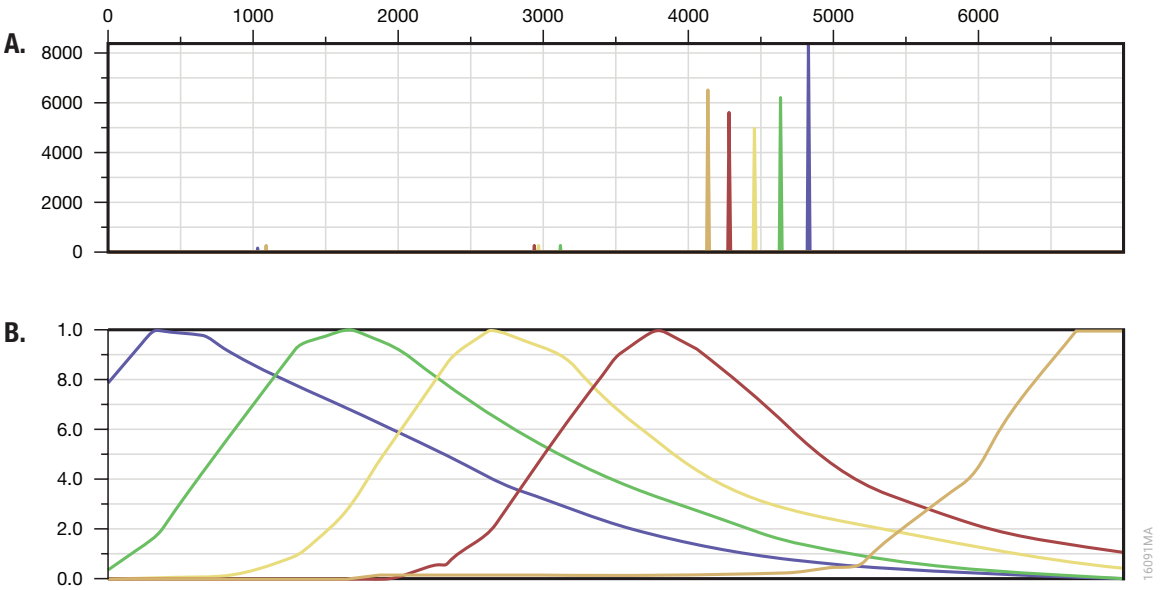


Figure 1. Representative data for the OncoMate™ 5C Matrix Standard. Panel A. Resolved fragment peaks. **Panel B.** Dye emission spectra.

10. Troubleshooting

For questions not addressed here, consult the capillary electrophoresis instrument user guide or contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**; e-mail: **genetic@promega.com**.

Symptoms	Causes and Comments
Capillary electrophoresis signal spike or dye-order error detected	Contaminants or crystal deposits were present in the polymer. Ensure newly added polymer is brought to room temperature following the manufacturer's instructions. Repeat the spectral calibration. If necessary, replace the polymer.
	Bubbles were present in the instrument fluidics. Consult the capillary electrophoresis instrument manual to determine how to clear the bubbles in the instrument fluidics, and then repeat the spectral calibration.
Spectral calibration failed or no signal was detected	An error occurred on the system computer. Reboot the capillary electrophoresis instrument and the instrument's computer following the manufacturer's instructions. Repeat the spectral calibration.
	Instrument was not adequately preheated. If required, ensure that the instrument oven was preheated to 60°C for at least 30 minutes prior to calibration. Repeat the spectral calibration.
	Instrument consumables were expired, or their quality was compromised. For best spectral calibration results, use fresh polymer, fresh buffers and a capillary array with fewer than 100 injections.
	An inappropriate dye set was selected for spectral calibration or the spectral calibration settings for the dye set were programmed incorrectly. Ensure that a 5-color dye set is used and that the analysis settings for the 5-color dye set are programmed accurately (see Table 1).
	Too few or incorrect fragment peaks were detected during spectral calibration. Carry-over from previous injection(s) was present, or matrix reagents were expired or were improperly stored. Repeat spectral calibration using properly stored or unexpired reagents, if applicable. Completing a blank (Hi-Di™ formamide only) injection prior to repeating the spectral calibration may be necessary.
	The OncoMate™ 5C Matrix Standard was prepared incorrectly. Prepare fresh diluted 5C Matrix Mix as described in Section 8, and perform a new spectral calibration.

10. Troubleshooting (continued)

Symptoms	Causes and Comments
Spectral calibration failed or no signal was detected (continued)	The OncoMate™ 5C Matrix Standard was expired or degraded due to improper storage. Verify the expiration date and storage conditions of the matrix standard. If necessary, repeat the spectral calibration using properly stored, unexpired reagents.
	One or more capillaries were blocked. Refill the capillary array and repeat the spectral calibration. Install a new capillary array if necessary.
	Matrix standard was too dilute. Matrix standard that is too dilute will cause low spectral calibration peak heights, which can cause spectral calibration failure. Repeat the spectral calibration, ensuring that the 5C Matrix Mix is vortexed sufficiently prior to use and that the proper ratio of diluted 5C Matrix Mix to Hi-Di™ Formamide is used. If necessary, increase the volume of diluted 5C Matrix Mix added to formamide during sample preparation.
	Matrix standard was too concentrated. Matrix standard that is too concentrated will result in excessive spectral calibration peak heights. Excessive peak heights may lead to bleedthrough or oversubtraction in other dye colors and spectral calibration failure. If necessary, decrease the volume of diluted 5C Matrix Mix added to formamide during sample preparation. Repeat the spectral calibration, ensuring that the 5C Matrix Mix is vortexed sufficiently prior to use and the proper ratio of 5C Matrix Mix to Hi-Di™ Formamide is used.
	Poor-quality formamide was used. The quality of formamide is critical. Use only Hi-Di™ Formamide with the OncoMate™ 5C Matrix Standard. Following first use, freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause formamide breakdown. Poor-quality formamide and formamide exposed to freeze-thaw cycles contain ions that compete with DNA during injection. This results in lower peak heights and reduced sensitivity during capillary electrophoresis.
	The capillary tips were not in contact with the matrix standard. Ensure that 15µl of formamide-matrix mixture was added to each well of the 96-well plate and that the plate was centrifuged sufficiently prior to starting the spectral calibration.

11. Related Products

Product	Size	Cat.#
OncoMate™ MSI Dx Analysis System	100 reactions	MD3140

[a]U.S. Pat. No. 9,139,868, European Pat. No. 2972229, and other patents pending.

[b]TMR-ET, CXR-ET and WEN dyes are proprietary.

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