TECHNICAL MANUAL

OncoMate™ 5C Matrix Standard

Instructions for Use of Product
MD4850

IVD

INSTRUCTIONS FOR USE OF PRODUCT
MD4850

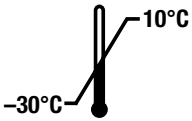
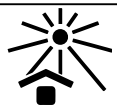
PROMEGA
2800 Woods Hollow Rd.
Madison, WI USA

Rev0
TM542

Promega

OncoMate™ 5C Matrix Standard

Symbols Key

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

1. Product Name

OncoMate™ 5C Matrix Standard
Part No. MD4850

Common Name
Fluorescently labeled DNA fragments, Matrix Standard, spectral calibration standard

2. Intended Use

The OncoMate™ 5C Matrix Standard is used for spectral calibration of the Applied Biosystems® 3500 Dx Genetic Analyzer configured with a 50cm 3500 Dx Capillary Array and POP-7® 3500 Dx Series polymer. The spectral calibration procedure generates a multicomponent matrix that is applied during the subsequent analysis of amplification products from the OncoMate™ MSI Dx Analysis System.

3. Summary and Explanation

The OncoMate™ MSI Dx Analysis System employs multiplex polymerase chain reaction (PCR) to generate fluorescent-dye-labeled DNA fragments spanning microsatellite markers. During analysis by capillary electrophoresis (CE), the dye-labeled DNA fragments are separated and detected by the instrument. Prior to analysis, the Applied Biosystems® 3500 Dx Genetic Analyzer must be calibrated with OncoMate™ 5C Matrix Standard to distinguish the fluorescent signals resulting from the set of 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 using the 'OncoMate_MSI' dye set, which is installed on the Applied Biosystems® 3500 Dx Genetic Analyzer using the OncoMate™ MSI Dx Assay Installer. The result of the spectral calibration is a multicomponent matrix, which is applied during sample detection to compensate for spectral overlap among the dyes and separate the raw fluorescent signals into individual dye signals.

For protocols to operate the Applied Biosystems® 3500 Dx Genetic Analyzer and recommendations on when to perform a new spectral calibration, please refer to the *Applied Biosystems® 3500 Dx Genetic Analyzer and 3500xL Dx Genetic Analyzer IVD User Guide*.

A spectral calibration must be generated for each individual Applied Biosystems® 3500 Dx Genetic Analyzer. A new spectral calibration must be run after any major maintenance on the system, such as changing the laser, calibrating or replacing the CCD camera, or changing the polymer type or capillary array. We also recommend that you perform a new spectral calibration after the instrument is moved to a new location or serviced by the manufacturer. In some instances, a software upgrade also may require a new spectral calibration.

4. Principles of the Procedure

During the data collection portion of a CE 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 in 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 analysis software must distinguish dye emission spectra.

A spectral calibration standard, or matrix standard, consists of fluorescently labeled DNA fragments that are analyzed during a spectral calibration. The CE 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

Σ 5

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 three 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.

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

5.3 Materials Not Provided

Laboratory Reagents

- Conditioning Reagent 3500 Dx Series (Applied Biosystems Cat.# 4409543; required when changing polymer types)
- Hi-Di™ Formamide 3500 Dx Series (Applied Biosystems Cat.# 4404307)

The use of Hi-Di™ Formamide 3500 Dx Series 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 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 CE 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

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Instruments and Accessories

- Applied Biosystems® 3500 Dx Genetic Analyzer (Thermo Fisher Cat.# A46344)
- 3500 Dx Capillary Array, 50cm (Thermo Fisher Cat.# 4404684)
- POP-7® Polymer 3500 Dx Series (Thermo Fisher Cat.# 4393709, 4393713)
- Anode Buffer Container 3500 Dx Series (Thermo Fisher Cat.# 4393925)
- Cathode Buffer Container 3500 Dx Series (Thermo Fisher Cat.# 4408258)
- MicroAmp® Optical 96-Well Reaction Plate with Barcode (Thermo Fisher Cat.# 4306737)
- 3500 Dx Series 96-Well Standard Retainer and Base Set (Thermo Fisher Cat.# 4410227)
- 3500 Dx Series Septa 96-Well (Thermo Fisher Cat.# 4410700)
- 3500 Dx Series Septa Cathode Buffer Container (Thermo Fisher Cat.# 4410716)

6. Preparation of the Applied Biosystems® 3500 Dx Genetic Analyzer

Spectral calibration for the OncoMate™ MSI Dx Analysis System uses the 'OncoMate_MSI' dye set, which is installed on the Applied Biosystems® 3500 Dx Genetic Analyzer using the OncoMate™ MSI Dx Assay Installer. This installation must occur prior to the first spectral calibration run. See the *OncoMate™ MSI Dx Analysis System Technical Manual* #TM543 for complete assay installation instructions.

OncoMate™ MSI Dx Analysis System amplification products are analyzed using a 50cm 3500 Dx Series Capillary Array and POP-7® 3500 Dx Series Polymer, and the corresponding spectral calibration must be performed using the same configuration. If required, use the Change Polymer Type wizard to install POP-7® 3500 Dx Series Polymer on the capillary electrophoresis instrument. Conditioning Reagent 3500 Dx Series is required when changing polymer type. Refer to the *Applied Biosystems® 3500 Dx Genetic Analyzer and 3500xL Dx Genetic Analyzer IVD User Guide* for additional information on performing spectral calibrations and instrument maintenance.

We recommend the use of a new capillary array, fresh polymer and fresh buffer for an optimal spectral calibration.

1. Before preparing the Matrix Standard, open the 3500 Series Data Collection Software, and select **Diagnostic Mode** upon login. Navigate to the Dashboard screen (Figure 1). Complete any instrument maintenance requested under Calendar Reminders. Under Consumables Information, ensure that consumables are not expired. Within the instrument, inspect the consumables to ensure that buffer levels are at their fill lines. Check the pump assembly for bubbles, and run the Remove Bubble wizard if needed.
2. Set the oven temperature to 60°C, and then select **Start Pre-Heat**. Preheat the oven for at least 30 minutes before starting a run.

7. Preparation of the Matrix Standard for Analysis

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 3500 Dx Series. Vortex for 10–15 seconds at maximum speed.

4. For the eight-capillary Applied Biosystems® 3500 Dx Genetic Analyzer, eight wells (A1 through H1 of a 96-well plate) are used for spectral calibration. Add 15µl of formamide-matrix mix (prepared in Step 3) to each of the eight wells in column 1 of a MicroAmp® Optical 96-Well Reaction Plate. Cover the plate with a 3500 Dx Series septa mat, 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.

5. Place the plate in the 96-Well Plate Base and cover with the Plate Retainer. Load the plate onto the Applied Biosystems® 3500 Dx Genetic Analyzer. Ensure that the oven is preheated to 60°C before starting the spectral calibration.

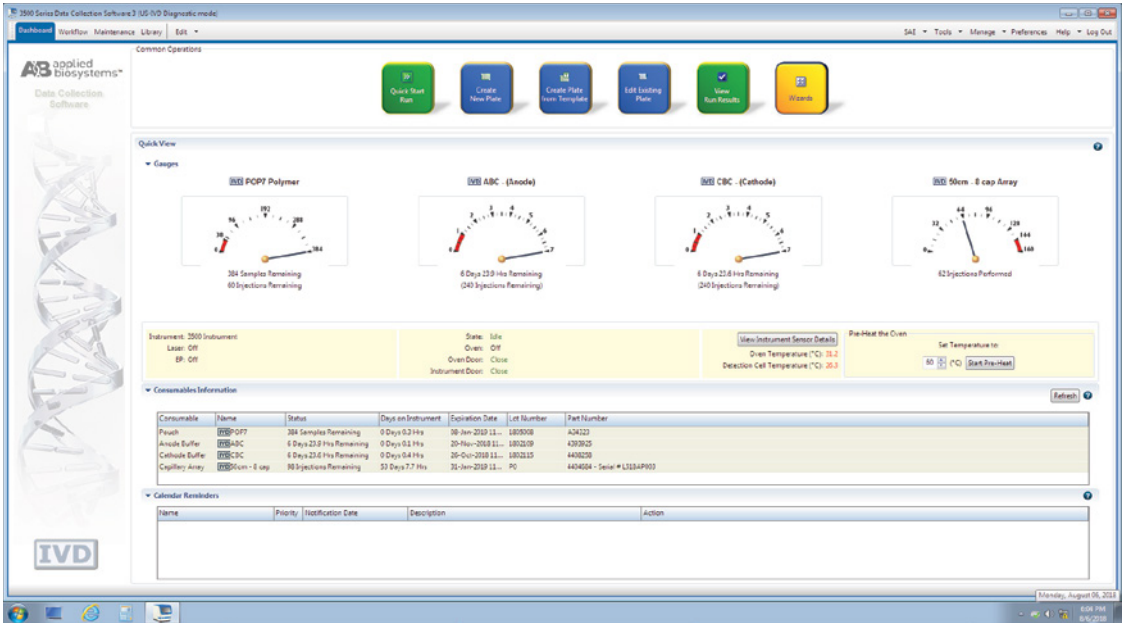

Figure 1. Dashboard on the Applied Biosystems® 3500 Dx Genetic Analyzer installed with POP-7® polymer, a 50cm array and Data Collection Software Version 3.2.

8. Spectral Calibration of the Applied Biosystems® 3500 Dx Genetic Analyzer

1. To perform the spectral calibration, open the 'Maintenance' tab in the menu bar, and then choose **Spectral** in the navigation pane.

2. Under Calibration Settings, choose **Matrix Standard** as the Chemistry Standard, **OncoMate_MSI** as the Dye Set and **A01** as the Starting Well. See Figure 2.

   Optional settings: When "Perform Run2/Run3 if Run1 Fails" is selected, the run will be repeated automatically up to three times if the spectral calibration fails. When "Allow Borrowing" is selected, the software automatically replaces information from failed capillaries with information from the adjacent passing capillary with the highest Quality Value. A single borrowed capillary is allowed for a passing spectral calibration run.

3. Click **Start Run**.

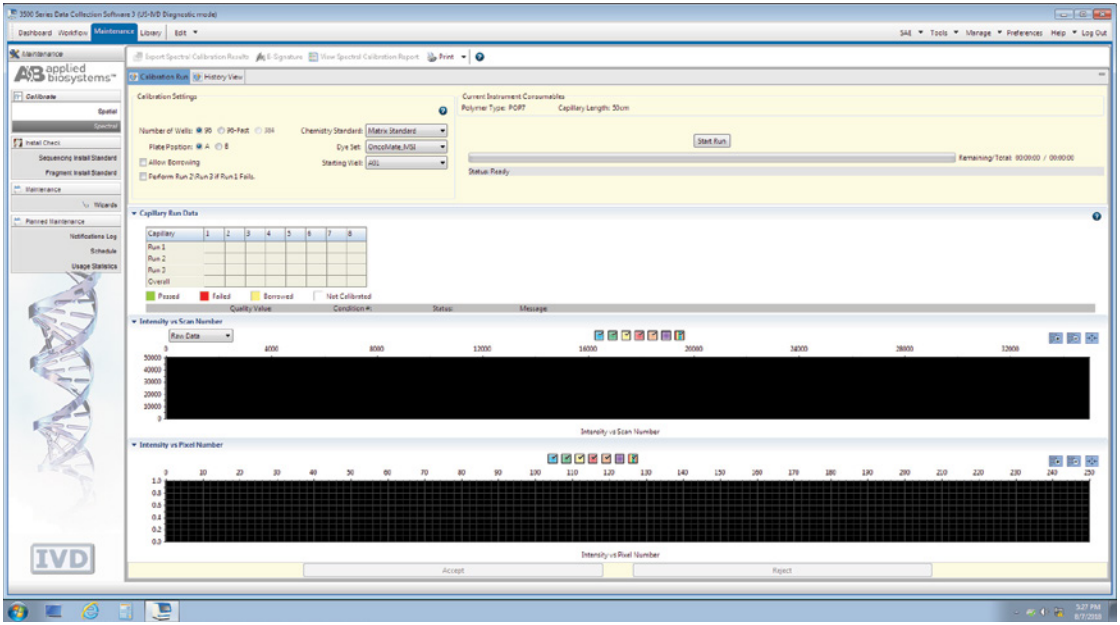

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Figure 2. Calibration Run window.

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 in the Capillary Run Data display, and inspect the spectral emission data in the Intensity vs Scan Number and Intensity vs Pixel Number displays (Figure 3). 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. Ensure that the order (left to right) of the dye signals in Intensity vs Pixel Number display is blue, green, yellow, red and orange.

2. If all capillaries passed, or if "Allow Borrowing" was selected and ≥7 capillaries passed, and if the corresponding emission data were displayed correctly, click **Accept** to accept the spectral calibration. Otherwise, click **Reject** to reject the spectral calibration, and then refer to Section 10, Troubleshooting.

For additional information on the interpretation of spectral calibration results, refer to the *Applied Biosystems® 3500 Dx Genetic Analyzer and 3500xL Dx Genetic Analyzer IVD User Guide*.

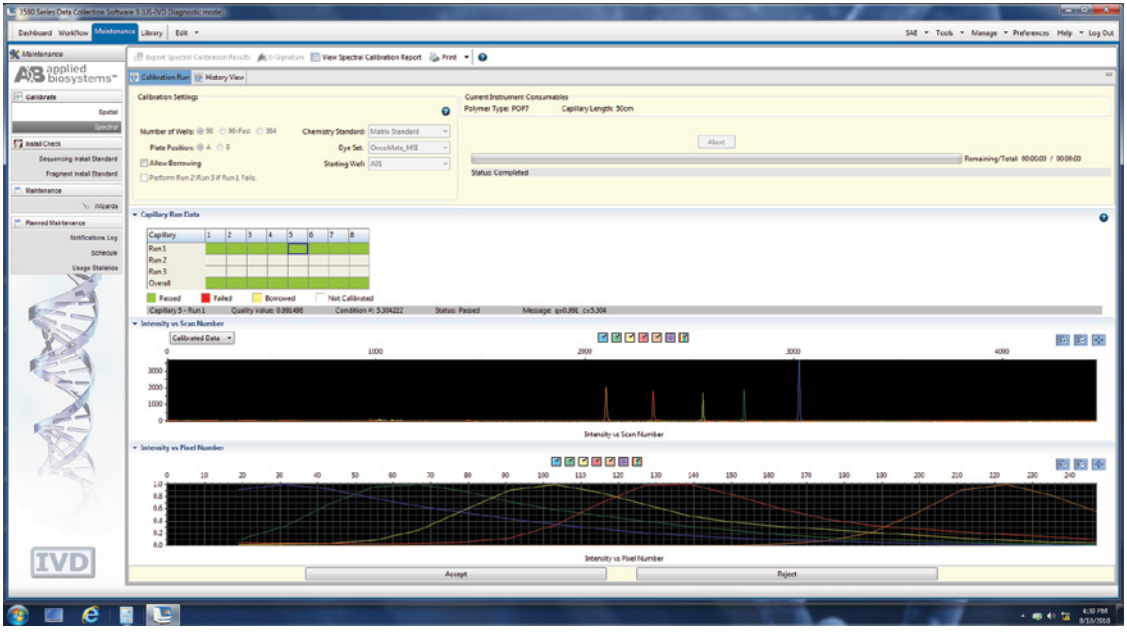

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Figure 3. Representative data for the OncoMate™ 5C Matrix Standard on the Applied Biosystems® 3500 Dx Genetic Analyzer using POP-7® Polymer for 3500 Dx Series and Data Collection Software Version 3.2.

10. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**; e-mail: **genetic@promega.com**

Symptoms	Causes and Comments
Data spikes or "Bad dye order detected" error	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. Run the Remove Bubble wizard 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 CE instrument and the instrument's computer following the manufacturer's instructions. Repeat the spectral calibration.
	Instrument was not adequately preheated. 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.
	Matrix standard was prepared incorrectly. Prepare fresh diluted 5C Matrix Mix as described in Section 7, and perfrom a new spectral calibration.
	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.
	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.
	One or more capillaries were blocked. Refill the capillary array, and repeat the spectral calibration. Installation of a new capillary array may be necessary.

Symptoms	Causes and Comments
Spectral calibration failed or no signal was detected (continued)	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. 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. If necessary, decrease the volume of diluted 5C Matrix Mix added to formamide during sample preparation.
	Poor-quality formamide was used. The quality of formamide is critical. Use only Hi-Di™ Formamide 3500 Dx Series 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.

For general information about spectral calibrations, refer to the *Applied Biosystems® 3500 Dx Genetic Analyzer and 3500xL Dx Genetic Analyzer IVD User Guide*.

11. Related Products

Product	Size	Cat.#
OncoMate™ MSI Dx Analysis System	100 reactions	MD2140
OncoMate™ MSI Dx Interpretive Software	1 each	MD4140

[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.