# PowerPlex® Matrix Standards for Use on the Spectrum CE System Technical Manual

Instructions for Use of Products DG4850, DG4900 and DG5010


TMD068 | REVISED 12/24

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All technical literature is available at: **www.promega.com/protocols/**

Visit the website to verify that you are using the most current version of this Technical Manual.

Email Promega Technical Services if you have questions on use of this system: **genetic@promega.com**

# 1 Description

Proper generation of spatial and spectral calibration files is critical to evaluate multicolor systems with the Spectrum CE System. Refer to the *Spectrum CE System Operating Manual* #TMD052 for the instrument maintenance schedule and instructions for installation of the capillary array, buffers and polymer pouch.

The PowerPlex® Matrix Standards (see Table 1) consist of DNA fragments labeled with different fluorescent dyes. Matrix standards are used to create spatial and spectral calibrations in a single calibration assay for each dye set. Fragments of the matrix standard are first used to create a spatial calibration of the array, then a spectral calibration of the dyes. Once generated, the spectral calibration file is applied during sample detection to calculate the spectral overlap and separate the raw fluorescent signals into individual color signals. A calibration must be generated for each individual instrument, and must be performed after the installation or reinstallation of a capillary array. A calibration should also be performed after any major maintenance on the system, such as changing the laser, replacing the camera or if a decrease in spectral separation is observed in the STR results. We also recommend running a calibration any time the optics window has been opened or the instrument is moved to a new location.

**Table 1. PowerPlex® Matrix Standards.**

| Matrix Standard | Dyes | Cat.# | STR Systems |
|---|---|---|---|
| PowerPlex® 8C Matrix Standard[a,b] | FL-8C, JOE-8C, AQA-8C, TMR-8C, CXR-8C,TOM-8C, WEN-8C, CCO-8C | DG5010 | PowerPlex® 35GY |
| PowerPlex® 6C Matrix Standard[a,c] | FL-6C, JOE-6C, TMR-6C, CXR-6C, TOM-6C, WEN | DG4900 | PowerPlex® Fusion 6C |
| PowerPlex® 5C Matrix Standard[d] | Fluorescein, JOE, TMR-ET, CXR-ET, WEN | DG4850 | PowerPlex® Fusion<br>PowerPlex® ESX/ESI 17 Fast<br>PowerPlex® ESX/ESI 16 Fast<br>PowerPlex® ESX 17<br>PowerPlex® ESI 17 Pro<br>PowerPlex® 21<br>PowerPlex® 18D<br>PowerPlex® Y23 |

# 2 PowerPlex® 8C Spectral Calibration

## 2.1 Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| PowerPlex® 8C Matrix Standard | 5 preps | DG5010 |

Not For Medical Diagnostic Use. Includes:

- 150µl 8C Matrix Mix
- 5 × 200µl Matrix Dilution Buffer

### Storage Conditions

Upon receipt, store all components at +2°C to +10°C. Store protected from light. We strongly recommend that the PowerPlex® 8C Matrix Standard be stored with post-amplification reagents. The PowerPlex® 8C Matrix Standard is light-sensitive; dilute the 8C Matrix Mix in the Matrix Dilution Buffer in the provided amber tube. Store the diluted 8C Matrix Mix at +2°C to +10°C for up to 1 week.

Do not freeze reagents.

### Materials to be Supplied by the User

- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- Spectrum Capillary Array, 8-Capillary (Cat.# CE2008)
- Spectrum Polymer4, 384 Wells (Cat.# CE2048) or
  Spectrum Polymer4, 960 Wells (Cat.# CE2040)
- Spectrum Buffer and Cathode Septa Mat Bundle (Cat.# CE2012)
- MicroAmp® Optical 96-Well Reaction Plate (0.2ml; Applied Biosystems)
- Septa Mat, 96-Well (Cat.# CE2696) or equivalent Applied Biosystems septa mat
- Hi-Di™ formamide (Applied Biosystems, Cat.# 4311320)

For additional information on performing calibration, refer to the *Spectrum CE System Operating Manual* #TMD052.

The quality of formamide is critical. Use only the recommended formamide. Freeze the formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at +2°C to +10°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

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. Always wear gloves and safety glasses when working with formamide.

**Notes:**

a. Only use MicroAmp® Optical 96-Well Reaction Plates (0.2ml).

b. Wear gloves when handling consumables and sample plate assembly.

## 2.2 Matrix Sample Preparation

1. Vortex the 8C Matrix Mix for 10–15 seconds prior to use. Add 10µl of the 8C Matrix Mix to one tube of the Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the date of dilution. The diluted 8C Matrix Mix can be stored for up to 1 week at +2°C to +10°C.
2. Vortex the diluted 8C Matrix Mix prepared in Step 1 for 10–15 seconds, then add 10µl to 500µl of formamide.
3. Vortex the diluted 8C Matrix Mix with formamide prepared in Step 2 for 10–15 seconds, then add 15µl to wells A1 through H1 of a 96-well plate. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles. Do not heat denature.

## 2.3 Assembling the Sample Plate

1. Place the 96-well plate into the Spectrum plate base, lining up the notch above well A12 with the notch on the base.
2. To complete the plate assembly, place the Spectrum plate retainer over the plate/base assembly, lining up the notch on the retainer with the notch on the plate and base. Verify that the retainer is locked in place (Figure 1).

**Figure 1. The Spectrum plate assembly.**

## 2.4 Instrument Preparation and Spatial/Spectral Calibration

These instructions are intended as a guide for running PowerPlex® Matrix Standards on the Spectrum CE System. They are not intended as comprehensive instructions for using the Spectrum CE System. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details on performing calibrations.

**Notes:**

a. We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration.

b. Do not calibrate with expired reagents. Expired reagents should be replaced before performing a calibration.

c. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details on installing consumables and instrument maintenance.

1. Select the **Consumables** indicator in the header. Ensure that the consumables are not expired and that adequate injections of the installed consumables remain.
2. Toggle the Oven indicator in the header to the **ON** position to preheat the oven to 60°C.

   **Note:** We recommend preheating the oven for at least 30 minutes prior to a run.
3. Navigate to the Maintenance Menu and select **Calibration**. Choose **Promega** from the Manufacturer drop-down menu. To perform a spectral calibration using the PowerPlex® 8C Matrix Standard, select the **START** button corresponding to "Promega 8-Dye (8C)". This will launch the Calibration wizard.

   **Notes:**

   a. If a run is in progress, the **START** button is deactivated. A calibration run can only be started when all other runs are complete.

   b. No calibration date and time is displayed in the dye set drop-down menu unless you have previously performed a calibration for that dye set. "(Uncalibrated)" is displayed after the dye set name.
4. Check the Message Center to verify that the drawer is not locked ("Drawer Locked" is not displayed in the Message Center.) Verify that the drawer handle light is illuminated indicating the drawer is unlocked and ready for plate loading.
5. Open the plate drawer.
6. Follow the instructions in the Calibration wizard to load the plate in the drawer, then select **NEXT** to close the wizard and display the calibration run screen for the selected dye set.
7. Select the number of injections to schedule for the calibration run. Up to three calibration injections can be run in succession.
8. Select a Spectral Calibration protocol, defined in the Protocols submenu (see Section 7.1 of the *Spectrum CE System Operating Manual* #TMD052 for more information).
9. Link the calibration run to the plate position by selecting **LINK** under the plate icon in the Plate Position indicator.
10. Select the green **Start** button in the header to start the calibration run.

Upon completing a calibration injection, the software processes the spatial calibration data. If the spatial calibration passes, the spectral calibration data is processed. The 'Calibration Status' column reflects the status and outcome of the calibration (Figure 2). A chasing yellow line indicates a calibration in progress. A red line indicates a failed calibration. A green line indicates a passing calibration.

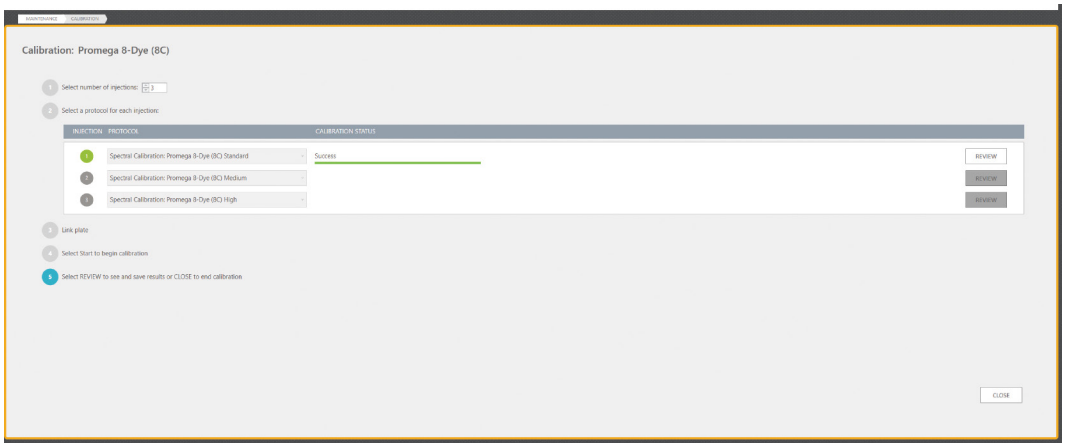


**Figure 2. First calibration injection success.**

If a calibration injection passes, any remaining injections are automatically canceled. If a calibration injection fails, the next scheduled injection begins, if applicable.

## 2.5 Results

1. Select the **REVIEW** button in the injection row to open the calibration review screen for that injection.

   **Note:** You must review and activate passing results to activate a calibration.

2. The calibration review screen can be used to evaluate the overall calibration status, Max Spectral Bleedthrough value, spectral quality flags, raw data and emission spectra for each capillary. If the calibration passed, the calibration review screen displays the data for each capillary in the form of a deconvoluted raw data plot on the left and the emission spectra on the right of the plot area (Figure 3).


**Figure 3. Passing calibration on the calibration review screen.**

3. Confirm that the number of distinct emission peaks for each capillary matches the required number of peaks as determined by the dye set (e.g., eight peaks for the Promega 8-Dye (8C) dye set). This value is displayed through spectral quality flags in the plate layout on the right side of the calibration review screen (see Figure 3 below the **Success** bar).

   **Note:** The minimum peak height for a dye-labeled fragment is 50 relative fluorescence units (RFU).

4. Confirm each capillary has met the passing criteria for spectral bleedthrough set in the 'Calibration' tab of the Preferences submenu (see Section 7.3 of *Spectrum CE System Operating Manual* #TMD052 for more information). The Max Spectral Bleedthrough value displayed is the highest bleedthrough percentage observed across the capillary array (see Figure 3 below the plate layout).

5. If the data of a single capillary has failed to meet the criteria, the calibration fails. Select **Failure Details…** below the calibration status to review the failure description and the spectral quality flags for each capillary in the plate layout. Depending on the reason for failure, you can adjust the analysis start point and reanalyze the calibration data.

   **Notes:**

   a. Refer to the *Spectrum CE System Operating Manual* #TMD052 for details on spectral quality flags and analysis start point adjusting.

   b. Refer to Section 5 for resolving failed spatial/spectral calibrations.

6. After reviewing the data for all capillaries, select **ACTIVATE** at the bottom right of the calibration review screen (Figure 3) to accept passing results and activate the calibration for the dye set. A confirmation window appears to verify that the calibration is active. Select **OK** to close the window and return to the 'Calibration Management' screen. Selecting **CLOSE** will return to the calibration run screen without activating the calibration.

   **Note:** The **ACTIVATE** button is not active if the calibration status is "Fail".

7. After returning to the 'Calibration Management' screen, the active passing calibration is displayed in the dye set drop-down menu by its run name and injection number (e.g., Promega 8-Dye (8C) (2022.09.1 13:58:26) #1). Selecting a calibration from the drop-down list sets it as the active calibration.

   **Note:** Passing calibrations remain in the drop-down list for the duration of an array installation period on an instrument. All calibrations are cleared from the software when an array is uninstalled.

# 3 PowerPlex® 6C Spectral Calibration

## 3.1 Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| PowerPlex® 6C Matrix Standard | 5 preps | DG4900 |

Not For Medical Diagnostic Use. Includes:

- 150µl 6C Matrix Mix
- 5 × 200µl Matrix Dilution Buffer

### Storage Conditions

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 PowerPlex® 6C Matrix Standard components at +2°C to +10°C, protected from light. We strongly recommend that the PowerPlex® 6C Matrix Standard be stored with post-amplification reagents. The PowerPlex® 6C Matrix Standard is light-sensitive; dilute the 6C Matrix Mix in the Matrix Dilution Buffer in the provided amber tube. Store the diluted 6C Matrix Mix at +2°C to +10°C for up to 1 week.

Do not refreeze the PowerPlex® 6C Matrix Standard components.

### Materials to be Supplied by the User

- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- Spectrum Capillary Array, 8-Capillary (Cat.# CE2008)
- Spectrum Polymer4, 384 Wells (Cat.# CE2048) or
  Spectrum Polymer4, 960 Wells (Cat.# CE2040)
- Spectrum Buffer and Cathode Septa Mat Bundle (Cat.# CE2012)
- Spectrum Plate Base & Retainer, 96-Well (Cat.# CE5004)
- MicroAmp® Optical 96-Well Reaction Plate (0.2ml; Applied Biosystems)
- Septa Mat, 96-Well (Cat.# CE2696) or equivalent Applied Biosystems septa mat
- Hi-Di™ formamide (Applied Biosystems, Cat.# 4311320)

For additional information on performing calibration, refer to the *Spectrum CE System Operating Manua*l #TMD052.

The quality of formamide is critical. Use only the recommended formamide. Freeze the formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at +2°C to + 10°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

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. Always wear gloves and safety glasses when working with formamide.

**Notes:**

a. Only use MicroAmp® Optical 96-Well Reaction Plates (0.2ml).

b. Wear gloves when handling consumables and sample plate assembly.

## 3.2 Matrix Sample Preparation

1. At the first use, thaw the 6C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at +2°C to +10°C, protected from light.
2. Vortex the 6C Matrix Mix for 10–15 seconds prior to use. Add 10µl of the 6C Matrix Mix to one tube of the Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the date of dilution. The diluted 6C Matrix Mix can be stored for up to 1 week at +2°C to +10°C.
3. Vortex the diluted 6C Matrix Mix prepared in Step 2 for 10–15 seconds, then add 10µl to 500µl of formamide.
4. Vortex the diluted 6C Matrix Mix with formamide prepared in Step 3 for 10–15 seconds, then add 15µl to wells A1 through H1 of a 96-well plate. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles. Do not heat denature.

## 3.3 Assembling the Sample Plate

1. Place the 96-well plate into the Spectrum plate base, lining up the notch above well A12 with the notch on the base.
2. To complete the plate assembly, place the Spectrum plate retainer over the plate/base assembly, lining up the notch on the retainer with the notch on the plate and base. Verify that the retainer is locked in place (Figure 4).

**Figure 4. The Spectrum plate assembly.**

## 3.4 Instrument Preparation and Spatial/Spectral Calibration

These instructions are intended as a guide for running PowerPlex® Matrix Standards on the Spectrum CE System. They are not intended as comprehensive instructions for using the Spectrum CE System. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details on performing calibrations.

**Notes:**

a. We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration.

b. Do not calibrate with expired reagents. Expired reagents should be replaced before performing a calibration.

c. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details on installing consumables and instrument maintenance.

1. Select the **Consumables** indicator in the header. Ensure that the consumables are not expired and that adequate injections of the installed consumables remain.
2. Toggle the Oven indicator in the header to the **ON** position to preheat the oven to 60°C.

   **Note:** We recommend preheating the oven for at least 30 minutes prior to a run.
3. Navigate to the Maintenance Menu and select **Calibration**. Choose Promega from the Manufacturer drop-down menu. To perform a spectral calibration using the PowerPlex® 6C Matrix Standard, select the **START** button corresponding to "Promega 6-Dye (6C)". This will launch the Calibration wizard.

   **Notes**:

   a. If a run is in progress, the **START** button is deactivated. A calibration run can only be started when all other runs are complete.

   b. No calibration date and time is displayed in the dye set drop-down menu unless you have previously performed a calibration for that dye set. "(Uncalibrated)" is displayed after the dye set name.
4. Check the Message Center to verify that the drawer is not locked ("Drawer Locked" is not displayed in the Message Center.) Verify that the drawer handle light is illuminated indicating the drawer is unlocked and ready for plate loading.
5. Open the plate drawer.
6. Follow the instructions in the Calibration wizard to load the plate in the drawer, then select **NEXT** to close the wizard and display the calibration run screen for the selected dye set.
7. Select the number of injections to schedule for the calibration run. Up to three calibration injections can be run in succession.
8. Select a Spectral Calibration protocol, defined in the Protocols submenu (see Section 7.1 of the *Spectrum CE System Operating Manual* #TMD052 for more information).
9. Link the calibration run to the plate position by selecting **LINK** under the plate icon in the Plate Position indicator.
10. Select the green **Start** button in the header to start the calibration run.

Upon completing a calibration injection, the software processes the spatial calibration data. If the spatial calibration passes, the spectral calibration data is processed. The 'Calibration Status' column reflects the status and outcome of the calibration (Figure 5). A chasing yellow line indicates a calibration in progress. A red line indicates a failed calibration. A green line indicates a passing calibration.

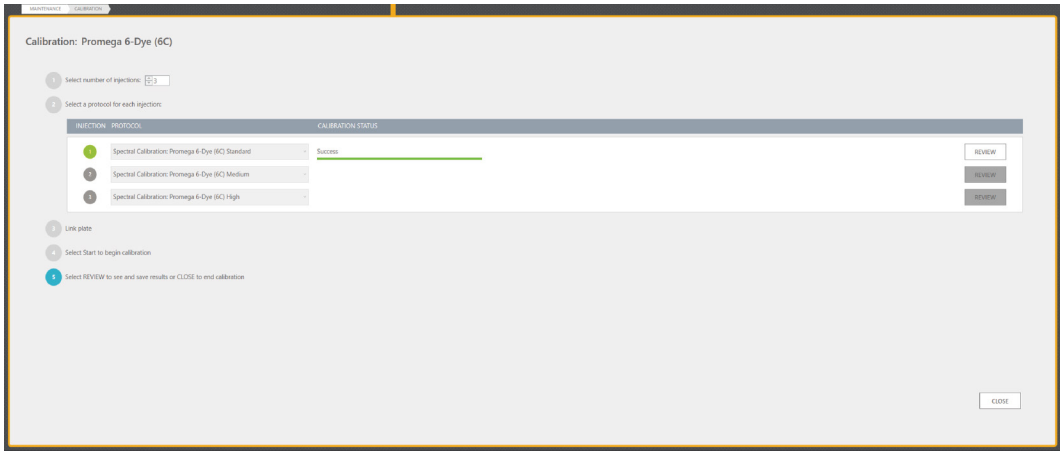


**Figure 5. First calibration injection success.**

If a calibration injection passes, any remaining injections are automatically canceled. If a calibration injection fails, the next scheduled injection begins, if applicable.

## 3.5 Results

1. Select the **REVIEW** button in the injection row to open the calibration review screen for that injection.

   **Note:** You must review and activate passing results to activate a calibration.

2. The calibration review screen can be used to evaluate the overall calibration status, Max Spectral Bleedthrough value, spectral quality flags, raw data and emission spectra for each capillary. If the calibration passed, the calibration review screen displays the data for each capillary in the form of a deconvoluted raw data plot on the left and the emission spectra on the right of the plot area (Figure 6).


**Figure 6. Passing calibration on the calibration review screen.**

3. Confirm that the number of distinct emission peaks for each capillary matches the required number of peaks as determined by the dye set (e.g., six peaks for the Promega 6-Dye (6C) dye set). This value is displayed through spectral quality flags in the plate layout on the right side of the calibration review screen (see Figure 6 below the **Success** bar).

   **Note:** The minimum peak height for a dye-labeled fragment is 50 relative fluorescence units (RFU). For optimal results, ensure peak heights are below 400,000RFU.

4. Confirm each capillary has met the passing criteria for spectral bleedthrough set in the 'Calibration' tab of the Preferences submenu (see Section 7.3 of *Spectrum CE System Operating Manual* #TMD052 for more information). The Max Spectral Bleedthrough value displayed is the highest bleedthrough percentage observed across the capillary array (see Figure 6 below the plate layout).

5. If the data of a single capillary has failed to meet the criteria, the calibration fails. Select **Failure Details…** below the calibration status to review the failure description and the spectral quality flags for each capillary in the plate layout. Depending on the reason for failure, you can adjust the analysis start point and reanalyze the calibration data.

   **Notes:**

   a. Refer to the *Spectrum CE System Operating Manual* #TMD052 for details on spectral quality flags and analysis start point adjusting.

   b. Refer to Section 5 for resolving failed spatial/spectral calibrations.

6. After reviewing the data for all capillaries, select **ACTIVATE** at the bottom right of the calibration review screen (Figure 6) to accept passing results and activate the calibration for the dye set. A confirmation window appears to verify that the calibration is active. Select **OK** to close the window and return to the 'Calibration Management' screen. Selecting **CLOSE** will return to the calibration run screen without activating the calibration.

   **Note:** The **ACTIVATE** button is not active if the calibration status is "Fail".

7. After returning to the 'Calibration Management' screen, the active passing calibration is displayed in the dye set drop-down menu by its run name and injection number (e.g., Promega 6-Dye (6C) (2022.01.12 13:58:26) #1). Selecting a calibration from the drop-down list sets it as the active calibration.

   **Note:** Passing calibrations remain in the drop-down list for the duration of an array installation period on an instrument. All calibrations are cleared from the software when an array is uninstalled.

# 4 PowerPlex® 5C Spectral Calibration

## 4.1 Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| PowerPlex® 5C Matrix Standard | 5 preps | DG4850 |

Not For Medical Diagnostic Use. Includes:

- 150µl 5C Matrix Mix
- 5 × 200µl Matrix Dilution Buffer

### Storage Conditions

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 PowerPlex® 5C Matrix Standard components at +2°C to +10°C, protected from light. We strongly recommend that the PowerPlex® 5C Matrix Standard be stored with post-amplification reagents. The PowerPlex® 5C Matrix Standard is light-sensitive; dilute the 5C Matrix Mix in the Matrix Dilution Buffer in the provided amber tube. Store the diluted 5C Matrix Mix at +2°C to +10°C for up to 1 week.

Do not refreeze the PowerPlex® 5C Matrix Standard components.

### Materials to be Supplied by the User

- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- Spectrum Capillary Array (8-Capillary) (Cat.# CE2008)
- Spectrum Polymer4, 384 Wells (Cat.# CE2048) or
  Spectrum Polymer4, 960 Wells (Cat.# CE2040)
- Spectrum Buffer and Cathode Septa Mat Bundle (Cat.# CE2012)
- Spectrum Plate Base & Retainer, 96-Well (Cat.# CE5004)
- MicroAmp® Optical 96-Well Reaction Plate (0.2ml; Applied Biosystems)
- Septa Mat, 96-Well (Cat.# CE2696) or equivalent Applied Biosystems septa mat
- Hi-Di™ formamide (Applied Biosystems, Cat.# 4311320)

For additional information on performing calibration, refer to the *Spectrum CE System Operating Manual* #TMD052.

The quality of formamide is critical. Use only the recommended formamide. Freeze the formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

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. Always wear gloves and safety glasses when working with formamide.

**Notes:**

a. Only use MicroAmp® Optical 96-Well Reaction Plates (0.2ml).

b. Wear gloves when handling consumables and sample plate assembly.

## 4.2 Matrix Sample Preparation

1. At the first use, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at +2°C to +10°C, protected from light.
2. Vortex the 5C Matrix Mix for 10–15 seconds prior to use. Add 10µl of the 5C Matrix Mix to one tube of the Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the date of dilution. The diluted 5C Matrix Mix can be stored for up to 1 week at +2°C to +10°C.
3. Vortex the diluted 5C Matrix Mix prepared in Step 2 for 10–15 seconds, then add 10µl to 500µl of Hi-Di™ formamide.
4. Vortex the diluted 5C Matrix Mix with formamide prepared in Step 3 for 10–15 seconds, then add 15µl to wells A1 through H1 of a 96-well plate. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles. Do not heat denature.

## 4.3 Assembling the Sample Plate

1. Place the 96-well plate into the Spectrum plate base, lining up the notch above well A12 with the notch on the base.
2. To complete the plate assembly, place the Spectrum plate retainer over the plate/base assembly, lining up the notch on the retainer with the notch on the plate and base (Figure 7). Verify that the retainer is locked in place on both sides of the plate, sitting evenly on top of the base.

**Figure 7. The Spectrum plate assembly.**

## 4.4 Instrument Preparation and Spatial/Spectral Calibration

These instructions are intended as a guide for running PowerPlex® Matrix Standards on the Spectrum CE System. They are not intended as comprehensive instructions for using the Spectrum CE System. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details on performing spectral calibration.

**Notes:**

a. We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration.

b. Do not calibrate with expired reagents. Expired reagents should be replaced before performing a spectral calibration.

c. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details on installing consumables and instrument maintenance.

1. Select the **Consumables** indicator in the header. Ensure that the consumables are not expired and that adequate injections of the installed consumables remain.
2. Toggle the Oven indicator in the header to the ON position to preheat the oven to 60°C.

   **Note:** We recommend preheating the oven for at least 30 minutes prior to a run.
3. Navigate to the Maintenance Menu and select **Calibration**. Choose **Promega** from the Manufacturer drop-down menu. To perform a spectral calibration using the PowerPlex® 5C Matrix Standard, select the **START** button corresponding to "Promega 5-Dye (5C)". This will launch the Calibration wizard.

   **Notes:**

   a. If a run is in progress, the **START** button is deactivated. A calibration run can only be started when all other runs are complete.

   b. No calibration date and time is displayed in the dye set drop-down menu unless you have previously performed a calibration for that dye set. "(Uncalibrated)" is displayed after the dye set name.
4. Check the Message Center to verify that the drawer is not locked ("Drawer Locked" is not displayed in the Message Center.) Verify that the drawer handle light is illuminated indicating the drawer is unlocked and ready for plate loading.
5. Open the plate drawer.
6. Follow the instructions in the Calibration wizard to load the plate in the drawer, then select **NEXT** to close the wizard and display the calibration run screen for the selected dye set.
7. Select the number of injections to schedule for the calibration run. Up to three calibration injections can be run in succession.
8. Select a Spectral Calibration protocol, defined in the Protocols submenu (see Section 7.1 of the *Spectrum CE System Operating Manual* #TMD052 for more information).
9. Link the calibration run to the plate position by selecting **LINK** under the plate icon in the Plate Position indicator.
10. Select the green **Start** button in the header to start the calibration run.

Upon completing a calibration injection, the software processes the spatial calibration data. If the spatial calibration passes, the spectral calibration data is processed. The 'Calibration Status' column reflects the status and outcome of the calibration (Figure 8). A chasing yellow line indicates a calibration in progress. A red line indicates a failed calibration. A green line indicates a passing calibration.

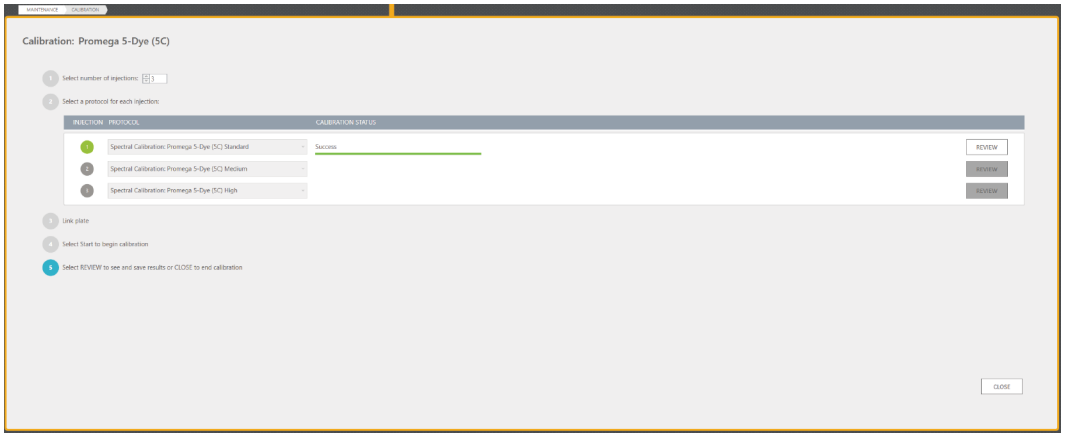


**Figure 8. First calibration injection success.**

If a calibration injection passes, any remaining injections are automatically canceled. If a calibration injection fails, the next scheduled injection begins, if applicable.

## 4.5 Results

1. Select the **REVIEW** button in the injection row to open the calibration review screen for that injection.

   **Note:** You must review and activate passing results to activate a calibration.

2. The calibration review screen can be used to evaluate the overall calibration status, Max Spectral Bleedthrough value, spectral quality flags, raw data and emission spectra for each capillary. If the calibration passed, the calibration review screen displays the data for each capillary in the form of a deconvoluted raw data plot on the left and the emission spectra on the right of the plot area (Figure 9).

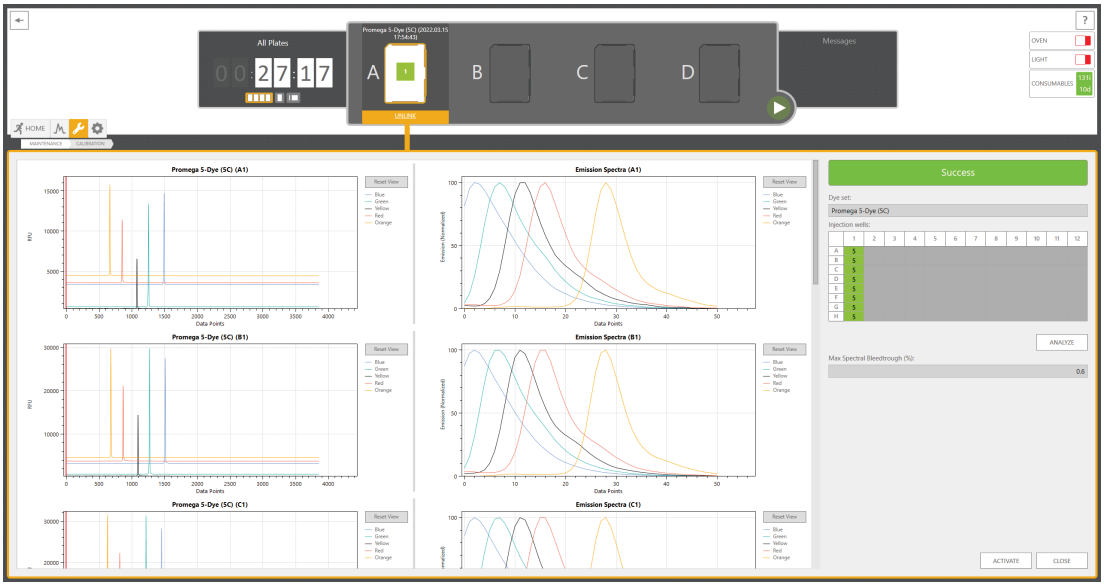


**Figure 9. Passing calibration on the calibration review screen.**

3. Confirm that the number of distinct emission peaks for each capillary matches the required number of peaks as determined by the dye set (e.g., five peaks for the Promega 5-Dye (5C) dye set). This value is displayed through spectral quality flags in the plate layout on the right of the calibration review screen (see Figure 9 below the Success bar).

   **Note:** The minimum peak height for a dye-labeled fragment is 50 relative fluorescence units (RFU). For optimal results, ensure peak heights are below 400,000RFU.

4. Confirm each capillary has met the passing criteria for spectral bleedthrough set in the 'Calibration' tab of the Preferences submenu (see Section 7.3 of *Spectrum CE System Operating Manual* #TMD052 for more information). The Max Spectral Bleedthrough value displayed is the highest bleedthrough percentage observed across the capillary array (see Figure 9 below the plate layout).

5. If the data of a single capillary has failed to meet the criteria, the calibration fails. Select **Failure Details…** below the calibration status to review the failure description and the spectral quality flags for each capillary in the plate layout. Depending on the reason for failure, you can adjust the analysis start point and reanalyze the calibration data.

   **Notes:**

   a. Refer to the *Spectrum CE System Operating Manual* #TMD052 for details on spectral quality flags and analysis start point adjusting.

   b. Refer to Section 5 for resolving failed spatial/spectral calibrations.

6 After reviewing the data for all capillaries, select **ACTIVATE** at the bottom right of the calibration review screen (Figure 9) to accept passing results and activate the calibration for the dye set. A confirmation window appears to verify that the calibration is active. Select **OK** to close the window and return to the 'Calibration Management' screen. Selecting **CLOSE** will return to the calibration run screen without activating the calibration.

   **Note:** The **ACTIVATE** button is not active if the calibration status is "Fail".

7. After returning to the 'Calibration Management' screen, the active passing calibration is displayed in the dye set drop-down menu by its run name and injection number (e.g., Promega 5-Dye (5C) (2022.01.12 13:58:26) #1). Selecting a calibration from the drop-down list sets it as the active calibration.

   **Note:** Passing calibrations remain in the drop-down list for the duration of an array installation period on an instrument. All calibrations are cleared from the software when an array is uninstalled.

# 5 Troubleshooting

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

| Symptoms | Causes and Comments |
|---|---|
| No peaks detected in any capillary or abnormal peak morphology for each capillary resulting in failing spatial calibration | Spectrum Capillary Array not installed correctly. Reinstall capillary array using the replace wizard. Confirm that the detection window is seated on the instrument. Wizard performs laser alignment to the array. |
| | Damaged Spectrum Capillary Array. Inspect capillary array for any damage or defects. If damage is observed, replace with a new Spectrum Capillary Array. |
| | Dust on capillary array window. Inspect capillary array window for dust or dirt. Gently remove dust with molecular-grade isopropanol and a lint-free wipe, allow to dry, reinstall and repeat calibration. |
| | Incomplete polymer fill or poor electrophoresis conditions. Repeat calibration. |
| No peaks detected in one or more dye channels for matrix standards | Incorrect matrix standard used for dye set. Confirm that the correct matrix standard was run with the correct dye set. |
| | Bubbles in the sample well. Centrifuge 96-well plate to remove air bubbles and repeat calibration. |
| | Confirm that eight wells of 96-well plate containing matrix standard have been loaded into wells A1 through H1. If matrix standard containing wells are not in these positions, they will not be injected and no peaks will be detected. |
| | Poor-quality formamide used with high conductivity. Prepare sample with fresh Hi-Di™ formamide. |
| | Matrix Mix was too dilute. Matrix Mix that is too dilute will result in low calibration peak heights (<50RFU above the baseline), which may result in spatial or spectral calibration failure. Increase the volume of diluted Matrix Mix added to the formamide during sample preparation. |

| Symptoms | Causes and Comments |
|---|---|
| No peaks detected in one or more dye channels for matrix standards (continued) | The cathode end of the Spectrum Capillary Array did not enter the sample, preventing electrokinetic injection of the matrix standard. Check the volume of samples. Volumes of sample as low as 10µl result in successful injection, but lower volumes will increase the likelihood for injection failures. If insufficient volume is present, increase the volume to the recommended 15µl and repeat calibration. If volume is sufficient and no peaks are detected, contact Promega Technical Services. |
| | Confirm that the matrix standard was prepared correctly. Check the matrix standard, reagent expiration date and storage conditions. |
| | Diluted Matrix Mix was stored longer than one week at 4°C or at the incorrect temperature. Prepare a fresh dilution. |
| Spectral calibration failed | Check the raw data of the failed capillaries. Look for signs of low or high peak heights, incorrect dye order for matrix standard (may indicate incompatible dye set used for matrix standard), high baseline noise, spikes or unexpected additional peaks that migrate before the matrix standard peaks. Adjust the analysis start point and reanalyze the spectral data as described in the *Spectrum CE System Operating Manual* #TMD052. |
| | Incorrect matrix standard used for dye set. Confirm that the correct matrix standard was run with the correct dye set. Possible failure messages: Insufficient peak count, Distinct emission mismatch, Out of order peaks. |
| | Peak present for matrix standard in raw data but too low for generating a spectral calibration (<50RFU above the baseline). Matrix Mix that is too dilute will result in low calibration peak heights, which may result in spectral calibration failure. Increase the volume of diluted Matrix Mix added to the formamide during sample preparation. |
| | Poor-quality formamide used with high conductivity. Prepare sample with fresh Hi-Di™ formamide. |
| | For best calibration results, use fresh polymer and fresh buffer. |
| | Unexpected peaks that migrate before the matrix standard peaks may indicate carryover from a previous injection. Adjust the analysis start point and reanalyze the spectral data as described in the *Spectrum CE System Operating Manual* #TMD052. Replace Spectrum ABC and CBC and the Spectrum Cathode Septa mat to eliminate any potential sources of contamination from a previous injection. |
| | Confirm that the matrix standard was prepared correctly. Check the matrix standard, reagent expiration date and storage conditions. |
| | Diluted Matrix Mix was stored longer than one week at 4°C or at the incorrect temperature. Prepare a fresh dilution. |

| Symptoms | Causes and Comments |
|---|---|
| Elevated spectral bleedthrough in one or more capillaries | If elevated spectral bleedthrough is observed in one or more capillaries after installing a new capillary array, reinstall the capillary array using the replace wizard. Confirm that the detection window is seated on the instrument correctly.<br>**Note:** It is necessary after uninstalling and reinstalling the capillary array to perform a new calibration before running samples. |
| Spectral calibration history does not display previously run calibration | Calibrations are reset when arrays are replaced. |

# 6 Related Products

## Spectrum CE System Accessories and Consumables

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| Spectrum Capillary Array, 8-Capillary | 1 each | CE2008 |
| Spectrum Polymer4 | 384 wells | CE2048 |
| | 960 wells | CE2040 |
| Spectrum Buffer | 2 pair | CE2001 |
| Spectrum Cathode Septa Mat | 10 each | CE2002 |
| Spectrum Buffer and Cathode Septa Mat Bundle | 1 each | CE2012 |
| Septa Mat, 96-Well | 10 each | CE2696 |
| Spectrum Plate Base & Retainer, 96-Well | 4 each | CE5004 |
| Spectrum Wash Solution | 1 each | CE2099 |

Not for Medical Diagnostic Use.

# 7 Summary of Change

The following change was made to the 12/24 revision of this document:

1. Replaced the instrument photo on the cover page.

[a]U.S. Pat. No. 9,139,868, European Pat. No. 2972229, Japanese Pat. No. 6367307 and other patents pending.

[b]AQA-8C, TMR-8C, CXR-8C, TOM-8C, WEN-8C and CCO-8C dyes are proprietary.

[c]TMR-6C, CXR-6C, TOM-6C and WEN dyes are proprietary.

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



Class 1 Laser Product.