PowerPlex® 6C Matrix Standard

INSTRUCTIONS FOR USE OF PRODUCT DG4900

This document is a quick protocol for experienced users to perform a spectral calibration for 6-color PowerPlex® Systems. For complete protocol information and troubleshooting tips see the *PowerPlex® 6C Matrix Standard Technical Manual* #TMD046, which is available online at: **www.promega.com/protocols/**

Instrument Preparation and Spectral Calibration Using the Applied Biosystems® 3500 and 3500xL Genetic Analyzers

Matrix Sample Preparation

1. Vortex the 6C Matrix Mix for 10–15 seconds prior to use.
2. Add 10µl of the 6C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds.
3. Add 10µl of the diluted 6C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.
4. For the Applied Biosystems® 3500 Genetic Analyzer, add 15µl of the 6C Matrix Mix with formamide prepared in Step 3 to wells A1 through H1 of the 96-well plate. Place the septa on the plate, and then briefly centrifuge the plate. Do not heat denature.

   For the Applied Biosystems® 3500xL Genetic Analyzer, add 15µl of the 6C Matrix Mix with formamide prepared in Step 3 to wells A1 through H3 of a 96-well plate. Place the septa on the plate, and then briefly centrifuge the plate. Do not heat denature.
5. Place the plate in the 3500 series 96-well standard plate base, and cover with the plate retainer.

Instrument Preparation

1. Set the oven temperature to 60°C. Select the Start Pre-Heat icon at least 30 minutes prior to the first injection.
2. To perform a spectral calibration for the Promega 6-color PowerPlex® Systems, go to the Maintenance tab, select “Spectral”, and under the Calibration Run tab, choose the appropriate fields: Choose “Matrix Standard” from the Chemistry Standard drop-down menu and the previously created Promega 6-color dye set (i.e., Promega J6) from the Dye Set drop-down menu.
3. Select “Start Run”.
4. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display and choose either “Accept” or “Reject”.

PowerPlex® 6C Matrix Standard

INSTRUCTIONS FOR USE OF PRODUCT DG4900

Instrument Preparation and Spectral Calibration Using the Applied Biosystems® 3130 and 3130xl Genetic Analyzers with Data Collection Software, Version 4.0, with the DC v4 6-Dye Module v1 License

Matrix Sample Preparation

1. Vortex the 6C Matrix Mix for 10–15 seconds prior to use.
2. Add 10µl of the 6C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds.
3. Add 10µl of the diluted 6C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.
4. For the Applied Biosystems® 3130 Genetic Analyzer, add 15µl of the 6C Matrix Mix with formamide prepared in Step 3 to wells A1 through D1 of the 96-well plate. Place the septa on the plate, and then briefly centrifuge the plate. Do not heat denature.

   For the Applied Biosystems® 3130*xl* Genetic Analyzer, add 15µl of the 6C Matrix Mix with formamide prepared in Step 3 to wells A1 through H2 of a 96-well plate. Place the septa on the plate, and then briefly centrifuge the plate. Do not heat denature.
5. Place the plate in the 3130 series 96-well standard plate base, and cover with the plate retainer.

Instrument Preparation

1. Set the oven temperature to 60°C, and preheat the oven for at least 15 minutes prior to the first injection.
2. In the Run Module Editor, select “New”. Select “SPECTRAL” in the Type drop-down list, and select “Spect36_POP4” in the Template drop-down list. Change the Data Delay Time to 400 and the Run Time to 800. Change the Injection Time to 6 seconds.
3. Make the following selections in the Protocol Editor:
   - “Spectral” in the Type drop-down list
   - “J6” in the DyeSet drop-down list
   - “POP4” for the polymer
   - “36” in the Array Length drop-down list
   - “Matrix Standard” for the chemistry
   - Select the spectral module you created in the previous step in the Run Module drop-down list.
   - Select “Edit Parameters”, and change the Minimum Quality Score (“Q value”) to 0.95.
   - Select “OK” in the “Edit Parameters” window, and select “OK” in the Protocol Editor
4. In the Plate Manager, create a new plate record as described in the instrument user’s manual. In the dialog box that appears, select “Spectral Calibration” in the Application drop-down list, and select “96-well” as the plate type. Add entries in the owner and operator windows, name the plate and select “OK.”
5. In the spectral calibration plate editor dialog box, place sample names in the appropriate cells.
6. In the Instrument Protocol column, select the appropriate protocol (e.g., Promega J6). Ensure that this information is present for each row that contains a sample name. Select “OK”.
7. Run your plate as described in the instrument user’s manual.
8. Upon completion of the run, check the status of the spectral calibration in the Event Log window.

Ordering and Technical Information
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