Plexor® HY System

INSTRUCTIONS FOR USE OF PRODUCTS DC1000 AND DC1001.

This document is a quick protocol for experienced users to quantify DNA samples using the Plexor® HY System. For complete protocol information and troubleshooting tips, see the *Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems Technical Manual* #TM293, *Plexor® HY System for the Stratagene Mx3000P® and Mx3005P™ Quantitative PCR Systems* #TM294 or *Plexor® HY System for the Bio-Rad iQ™5 Real-Time PCR Detection System* #TM296. These technical manuals are available online at: **www.promega.com/protocols/**

Before using the Plexor® HY System with the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems and Bio-Rad iQ™5 Real-Time PCR Detection System, you must generate a passing spectral calibration using the Plexor® Calibration Kit, Set A (Cat.# DC1500). See the appropriate Plexor® HY System technical manual for more information.

Serial Dilution of the Plexor® HY Male Genomic DNA Standard

1. Ensure that the Plexor® HY Male Genomic DNA Standard was stored at 2–10°C overnight before first use. After the first use, store the DNA standard 2–10°C; do not freeze and thaw. Vortex the Plexor® HY Male Genomic DNA Standard at high speed for 10 seconds.
2. Prepare fresh serial dilutions of the Plexor® HY Male Genomic DNA Standard as described below. Vortex each dilution for 10 seconds before removing an aliquot for the next dilution. Change pipette tips between dilutions.

DNA Concentration	Volume of Plexor® HY Male Genomic DNA Standard	Volume of TE^{-4} Buffer
50ng/µl	Use undiluted Plexor® HY Male Genomic DNA Standard	0µl
10ng/µl	10µl of undiluted Plexor® HY Male Genomic DNA Standard	40µl
2ng/µl	10µl of 10ng/µl dilution	40µl
0.4ng/µl	10µl of 2ng/µl dilution	40µl
0.08ng/µl	10µl of 0.4ng/µl dilution	40µl
0.016ng/µl	10µl of 0.08ng/µl dilution	40µl
0.0032ng/µl	10µl of 0.016ng/µl dilution	40µl

Plexor® HY Reaction Setup

This protocol assumes 2µl of template per reaction. To amplify a larger template volume, refer to the appropriate Plexor® HY System technical manual.

1. Thaw the Plexor® HY 2X Master Mix, Plexor® HY 20X Primer/IPC Mix and Water, Amplification Grade.
2. Vortex the Plexor® HY 2X Master Mix and Plexor® HY 20X Primer/IPC Mix for 10 seconds.
3. Determine the number of reactions to be set up, including the no-template control reactions. Add 1–3 reactions to this number.

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4. Prepare the reaction mix by combining Water, Amplification Grade, Plexor® HY 2X Master Mix and Plexor® HY 20X Primer/IPC Mix as indicated below.

Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Plexor® HY 2X Master Mix	10µl				
Water, Amplification Grade	7µl				
Plexor® HY 20X Primer/IPC Mix	1µl				
Total volume	**18µl**				

5. Vortex for 10 seconds to mix. Do not centrifuge after vortexing.
6. Add 18µl of reaction mix to the appropriate wells of an optical-grade PCR plate.
7. Add 2µl of TE^{-4} buffer to the no-template control reactions.
8. Add 2µl of DNA standard or unknown sample to the appropriate wells.
9. Seal the plates, and centrifuge briefly.

Starting a Thermal Cycling Run

1. Set up the instrument as directed in the appropriate Plexor® HY System technical manual. Highlight only the wells in use on the plate map when assigning sample types.
2. Program the instrument or choose an existing thermal cycling program with the thermal cycling conditions shown below.

Step	Temperature	Time	Number of Cycles
Initial denaturation	95°C	2 minutes[1]	1
Denaturation	95°C	5 seconds	38 cycles
Annealing and extension	60°C	35 seconds[2]	
Melt temperature curve	See Note 3.		

[1]The initial denaturation time for the Bio-Rad iQ™5 Real-Time PCR Detection System is 30 seconds.

[2]The annealing and extension time for the Stratagene Mx3000P® and Mx3005P™ Quantitative PCR Systems is 40 seconds.

[3]For the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, use the default Dissociation Function settings. For the Stratagene Mx3000P® and Mx3005P™ Quantitative PCR Systems, use an initial temperature of 65°C with a 0.6°C increase and 40-second hold each cycle. For the Bio-Rad iQ™5 Real-Time PCR Detection System, use the default Melt Curve settings.

3. Place the PCR plate in the instrument, and immediately begin thermal cycling.

Preliminary Data Analysis and Data Export

After thermal cycling, data must be exported from the instrument software and imported into the Plexor® Analysis Software. Note that some real-time PCR instruments require preliminary analysis or processing of the raw data before export. When exporting data, be sure to export both the amplification data and melt/dissociation data.

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Data Analysis

1. Launch the Plexor® Analysis Software.
2. Import the data files.
3. Use the well selector in the PCR Curves tab of the Analysis Desktop to select and define each well or group of wells. Define the DNA standards by highlighting wells that contain DNA standards, selecting the Create Dilution Series icon, confirming that the series selected is "Vertical Series" and "Decreasing" and entering 50 for the starting concentration and 5 for the dilution factor. Define the no-template control reactions by highlighting wells that contain no-template controls and selecting the NTC icon.
4. Assign or edit sample names for the unknown samples in the Sample Names tab.
5. In the PCR Curves tab, adjust the expected target melt temperature and target melt temperature range.
6. Generate a standard curve for the autosomal channel (FL tab) by selecting all samples and DNA standards and then selecting "Add Standard Curve". Repeat for the Y channel (CO560 tab).
7. Select the Standard Curves tab to view the standard curves. DNA concentrations for all samples are displayed in the table next to the standard curve graph and in the sample details report.
8. If desired, use the normalization tool (generate a forensic report) to help determine which samples are suitable for analysis, which samples require dilution, which samples may need repurification and how much sample should be added to your autosomal or Y-STR amplifications.
   a. Select "Set Normalization and IPC Parameters" in the Forensics menu. In the dialog box that appears, be sure that the box next to "disable volume normalization (show concentrations and C_q only)" is not checked.
   b. Confirm that the appropriate autosomal and Y-STR amplification parameters are entered in the autosomal STRs, Y-STRs and Internal PCR Control (IPC) tabs.
   c. Select "OK".
   d. The Forensics report is displayed. To export the quantification data, select "Export 96-well Concentration" in the Forensics menu. Select "Autosomal Concentrations" for the autosomal results, enter a file name and select "Save". Select "Y Concentrations" for the Y results, enter a file name and select "Save".

For routine analysis, a master template may be defined and used to incorporate frequently used run, analysis and forensic parameters. Refer to the appropriate Plexor HY® System technical manual for additional information.

For complete protocol information see the appropriate Plexor HY® System technical manual, which is available online at: **www.promega.com/protocols/**

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Printed in USA. 8/15. Part# 9FB191