
Bio-Rad Laboratories

2000 Alfred Nobel Dr.
Hercules, CA 94547

1-800-424-6723



Bio-Plex™ *Validation Kit 3.0*

Instruction Manual

Catalog # 171-203000

For use with Bio-Plex Manager Software Version 3.0 and MCV plate II

or

For use with Bio-Plex Manager Software Version 2.0 and MCV plate

For technical service, call your local Bio-Rad office, or
in the US, call 1-800-4BIORAD (1-800-424-6723)

For research use only. Not for diagnostic procedures.

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Table of Contents

Section 1	Introduction	1
Section 2	Product Description	2
Section 3	Specifications	3
Section 4	Storage and Handling	3
Section 5	Principle of Optics Validation	4
Section 6	Principle of Reporter Channel Validation	4
Section 7	Principle of Classify Validation	7
Section 8	Principle of Fluidics Validation	8
Section 9	Procedure for Bio-Plex Manager 3.0/MCV plate II ..	9
9.1	One-Step Procedure for all Validation Parameters	9
9.2	Validation of Optics Alignment	11
9.3	Validation of Fluidics Integrity	13
9.4	Validation of Reporter Channel Performance	15
9.5	Validation of Classify Efficiency	16
9.6	Generating a Validation Report	18
9.7	Validation Report Example	19
Section 10	Procedure for Bio-Plex Manager 2.0/MCV plate ..	21
10.1	Validation of Optics Alignment	21
10.2	Validation of Reporter Channel Performance	23
10.3	Validation of Classify Efficiency	25
10.4	Validation of Fluidics Integrity	26
10.5	Generating a Validation Report	28
10.6	Validation Kit Worksheet and Report Form Examples	28
Section 11	Troubleshooting Guide	32
Section 12	Ordering Information	32
Section 13	Reference	33

Section 1

Introduction

Qualification of analytical instruments is a formal process of documenting that an instrument is fit for its intended use and that it is kept maintained and calibrated. The Bio-Plex validation kit is used for operational qualification (OQ) of the Bio-Plex protein array system. The validation kit is designed to validate the operation of all of the primary components of the system and is a valuable tool that allows the user to discriminate between assay and instrumentation problems.

The Bio-Plex validation kit consists of beads to evaluate the following components of the Bio-Plex protein array system: 1) optics alignment, 2) integrity of fluidics, 3) reporter channel performance, and 4) classify efficiency. A brief definition of the parameter and the principle of each procedure is described, along with complete procedures for evaluating each of the primary components. An explanation of the potential impact of each process on a typical Bio-Plex cytokine assay is included to assist the user in assay troubleshooting and development.

NOTE: Bio-Plex Manager 3.0 requires MCV plate II (Cat# 171-203031) for use with this Validation Kit, see Section 9.

Bio-Plex Manager 2.0 requires MCV plate (Cat# 171-203030) for use with this kit, see Section 10. Bio-Plex Manager 2.0 does not allow use of fluidics 1 and fluidics 2 beads. This step is omitted, follow Section 10 instructions.

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Section 2

Product Description

The following reagents are included in the Bio-Plex validation kit:

Reagent	Quantity
Optics validation bead set: Optics beads 1 and 2 (1×10^5 beads/ml)	2 x 10 ml black bottles
Fluidics validation bead set: Fluidics bead 1 (1×10^5 beads/ml)	1 x 10 ml black bottle
Fluidics bead 2	1 x 10 ml black bottle
Reporter validation bead set: Reporter blank, 1, 2, 3, 4, and 5 (1×10^5 beads/ml)	6 x 10 ml white bottles
Classify validation bead set: Classify bead 34, 38, 54, 73, 77 (1×10^5 beads/ml)	5 x 10 ml black bottles

NOTE: The validation kit must be used following GAL2 calibration using the RP1 high target value. Validation should be performed immediately following calibration.

The following materials are required but not supplied:

Bio-Plex MCV plate

Bio-Rad catalog #171-203031 MCV plate II, use with Bio-Plex Manager 3.0,
Bio-Rad catalog #171-203030 MCV plate, use with Bio-Plex Manager 2.0

Bio-Plex Protein Array System

Bio-Rad catalog #171-000001, 171-000003, or 171-000005

Bio-Plex Calibration Kit

Bio-Rad catalog #171-203060

mini vortexer
sterile distilled water
70% isopropanol
10% bleach
bulb pipets

Section 3

Specifications

General specifications for the validation kit are listed below. Certain specifications for the Bio-Plex validation kit may differ from lot to lot. For a complete listing of the current specifications, please refer to the package insert provided with your validation kit.

Parameter	Specification
Optics Validation	
DD mean	See insert
CL1 mean	See insert
CL1 CV% (Coefficient of Variable)	3–7%
CL2 mean	See insert
CL2 CV%	4–8%
RP1 mean	See insert
RP1 CV%	5–10%
Fluidics Validation	
% carryover	< or = 4.0%
Reporter Validation	
Dynamic range	4.15–4.28
Linearity	>0.995
Slope of response	0.0593–0.0799
Accuracy of response	>90%
Sensitivity	<200 MESF
Classify Validation	
Classify Bead 34, 38, 54, 73, 77	>80.0%
DD Efficiency	
% Efficiency	> or = 75%

Section 4

Storage and Handling

The Bio-Plex validation kit beads are stable if stored at 4°C protected from light. When using the Bio-Plex validation kit, remove beads from 4°C storage and dispense into the MCV plate. Return to 4°C storage immediately following use to preserve shelf life. All components are guaranteed for 6 months from the date of purchase when stored as specified in this manual.

Section 5

Principle of Optics Validation

Principle

The Bio-Plex array reader is a laser-based fluorescence detection system containing sensitive optics components. Alignment of the laser/optics system is critical for optimal instrument performance. A method for the assessment of the optics alignment is included in the validation kit. Acceptable specifications for the alignment procedure are listed in the product insert.

Impact on Assay Performance

The alignment of the optics bench of the Bio-Plex array reader is critical for proper assay performance. Misalignment of the reporter optics path can result in 1) reduced assay sensitivity or 2) poor well-to-well assay precision. Misalignment of the classification optics path can lead to 1) increased read times or 2) misclassification of one assay into another, leading to false positive or negative results. Correlation studies have been performed to determine the direct effect of misalignment on assay performance.

Section 6

Principle of Reporter Validation

Principle

The reporter (RP1) channel is the fluorescence channel used for assay quantitation (See Bio-Plex system hardware manual for more information regarding the principle of Bio-Plex technology). Therefore, validation of this component of the Bio-Plex system is a critical part of operational qualification. R-phycoerythrin (R-PE) is the primary reporter molecule used in Bio-Plex assays. A series of beads dyed with varying intensities of a fluorochrome spectrally matched to R-phycoerythrin are used for this procedure. Each of the reporter beads has been assigned a specific intensity value corresponding to the number of fluorescent R-PE molecules. These units of fluorescent measure are known as molecules of equivalent soluble fluorescence (MESF). MESF units allow direct correlation of instrument performance to a typical assay using R-PE as the indicator molecule. The primary reporter channel performance parameters are as follows: dynamic range, linearity, accuracy of reporter channel response, sensitivity, and slope of the response. Each of these parameters is related directly to the performance of the Bio-Plex array reader and has defined acceptable specifications. Definitions for the parameters and the applicability to a typical assay performed on the Bio-Plex array reader are listed below. If any of the parameters are not within the specified range, contact Bio-Rad Technical support for assistance.

Dynamic Range of Reporter Channel

Definition

The dynamic range is the calculated number of decades covered by the log amplifier from the slope and the histogram scale. The available range of channels on the Bio-Plex array reader is 4.5 log amp decades or 32,767 relative linear channels. The acceptable dynamic range of fluorescence measured by the Bio-Plex array reader using the Bio-Plex reporter beads is 4.15–4.28.

Impact on Assay Performance

The dynamic range of the Bio-Plex array reader is 4.5 log amp decades or 32,767 relative linear channels. It is desirable for the range of the instrument to be greater than the range of an assay. If the dynamic range of the instrument is less than that of an assay, the range of quantifiable analyte may be limited. This parameter will guide the user in defining the instrument versus the assay dynamic range limitations.

Linearity of Reporter Channel

Definition

The reporter validation bead set is utilized to construct a plot where the reporter channel median fluorescence intensity values are plotted against the corresponding assigned MESF values. Instrument linearity is expressed as the coefficient of determination or R-squared (R^2) value. The R^2 value must be >0.995 .

Impact on Assay Performance

The linearity of the instrument response may directly affect a typical standard or calibration curve in a Bio-Plex assay, thereby impacting the unknown values extrapolated from that curve. If the R^2 value is not within acceptable limits, it may be necessary to realign the optics or check the response of the reporter photomultiplier tube.

Accuracy of Reporter Channel Response

Definition

The accuracy of the reporter channel response is a more stringent measurement of the linearity than the R^2 value. Simply stated, the accuracy of the reporter channel response is the percent difference that the regression line is away from the actual MESF value data points. The desired accuracy value is $>90\%$.

Impact on Assay Performance

Since accuracy is also a measurement of the linearity of the instrument response, the same principles that apply to linearity also apply to accuracy of the reporter channel response. Accuracy values $<90\%$ could impact assay performance. The accuracy data is evaluated in combination with optics alignment to determine if the Bio-Plex array reader will perform according to specifications. It is possible for the accuracy value to fall out of specification before the linearity parameter. This is expected due to the fact that the accuracy parameter is a more sensitive measurement of linearity than the R^2 value. These data are correlated with optics alignment data as well as assay performance to determine when the array reader will not perform according to specifications.

Slope of the Reporter Channel Response

Definition

The slope of the regression line resulting from the plotting of reporter channel mean fluorescent values against assigned reporter channel validation bead MESF values is related to the dynamic range of the instrument. The slope of the regression line is a function of the response of the reporter channel photomultiplier tube. The acceptable range for the slope is 0.0593–0.0799.

Impact on Assay Performance

The slope of the regression line is directly related to the dynamic range of the instrument. The slope yields direct information about the response of the photomultiplier tube. If the photomultiplier tube signal saturates at low fluorescence values, the dynamic range of the instrument is affected. The slope of the line impacts the dynamic range and the range in turn impacts the quantifiable range of an assay. If the validation kit yields a value for the slope that is not within specifications, assay results could be adversely affected.

Sensitivity of Reporter Channel

Definition

Every instrument has an inherent level of noise due primarily to the electronics. The sensitivity of the Bio-Plex array reader is defined as the lowest detectable signal above instrument noise. Noise can be attributed to the laser, the photomultiplier tube, the amplification electronics or the fluidics. The acceptable sensitivity using the Bio-Plex array reader is <200 MESF.

Impact on Assay Performance

The sensitivity using the Bio-Plex validation kit is expressed in terms of MESF. The fluorescence is traceable to R-PE, the primary molecule used in Bio-Plex assays. The typical background or zero standard of a Bio-Plex cytokine assay falls at a median fluorescence intensity of 100. The background of a "blank" bead from the validation kit exhibits a median fluorescence intensity of 8. A plot of the median RP1 fluorescence intensity versus the MESF units illustrates that the instrument is approximately 10 times more sensitive than a Bio-Plex cytokine assay (See Figure 1). This is a desired result, as the sensitivity of the instrument should not directly limit assay sensitivity.

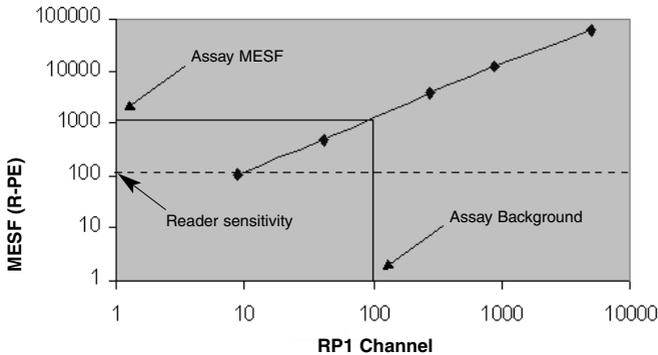


Fig. 1. Assay vs. instrument sensitivity.

Section 7 Principle of Classify Validation

Principle

Bio-Plex technology relies on the ability of the Bio-Plex array reader to discriminate between assay beads impregnated with varying ratios of 2 fluorescent dyes. This is the concept whereby multiplexing within a single well may occur. The periodic evaluation of the classify efficiency is necessary to complete the Bio-Plex array reader qualification process. A series of beads with varying ratios of the classification dyes are analyzed on the Bio-Plex array reader and the efficiency of multiplexing is quantitated. A classify efficiency of >80% is required for optimal results. DD Efficiency is a measure of the percentage of the Classify beads that fall within the DD Gates. Greater than 75% of the beads should fall within the gates for optimal results.

Impact on Assay Performance

Inefficient classification of beads may have several potential effects on an assay. If a bead region exhibits a classify efficiency of less than 80%, the read time of a 96-well plate may be increased. The Bio-Plex array reader tabulates a specified number of defined events in each region for each well sampled. If the percentage of beads within a specific region is low, the time required to count is increased, therefore the total time to read an entire plate is prolonged. Extremely prolonged assay read times could impact well-to-well precision, since the kinetics of a sandwich assay, for example, are not 100% stable over a period of 3–5 hrs. Another potential impact of inefficient classification is the misclassification of one assay bead into another bead region. This could yield false positive or negative results for a particular assay. A DD efficiency value less than 75% may increase the read time of the assay and affect results in the same manner as a low classify efficiency.

Section 8

Principle of Fluidics Validation

Principle

The fluidics system of the Bio-Plex suspension array reader requires routine maintenance to prevent clogging and other malfunctions. Strict adherence to the maintenance procedures is mandatory for optimal instrument performance. An assessment of the integrity of the fluidics is automatically performed in the Fluidics Validation procedure. In the fluidics validation test, a sample of beads is analyzed followed by a sample of buffer to assess the carryover of beads from one well to another. This procedure should be performed once per week to ensure that assay results are not adversely affected. The fluidics path, including the sample needle must be completely free of debris and excess beads for optimal array reader performance.

Impact on Assay Performance

If a system is exhibiting a high level of carryover, due to valve malfunction or partially clogged sample needle, a significant percentage of beads may be carried over from one well to another. This phenomenon may adversely affect the median fluorescent intensity values. For example, if a well with a high median fluorescent intensity (FI) is read immediately prior to a well with a low median FI, the signal in the well with the low fluorescent intensity may shift upward. This phenomenon only occurs in extreme cases since the median fluorescent intensity statistic is robust and is not easily shifted by the introduction of a population of beads with a significantly different median FI.

Section 9

Procedure for Bio-Plex Manager 3.0 and MCV plate II

Introduction

This section provides instructions for use of the Bio-Plex Validation Kit 3.0 with Bio-Plex Manager version 3.0 and MCV plate II. Bio-Plex Manager now provides a fully automated validation routine that sequentially performs all the validation tests without further user intervention. To perform all validations in a single step, follow instructions in Section 9.1. If you wish to perform an individual validation routine, these instructions are provided in Sections 9.2–9.5.

9.1 One-Step Procedure for all Validation Parameters

Procedure

1. Turn on the Bio-Plex array reader, microplate platform, and computer as specified in the Bio-Plex hardware and Bio-Plex Manager user manuals.
2. Perform start-up procedure as directed.
3. Calibrate Bio-Plex array reader using CAL 1 and CAL2 beads found in the Bio-Plex Calibration Kit according to the Bio-Plex Manager software manual.

Note: Be sure to calibrate immediately before validation. Use the High RP1 Target for CAL2 calibration.

4. Remove all validation bead sets from 4°C storage and vortex each and every bottle for 30 sec. This is very important for proper validation.

5. Select Instrument from the main menu. Select Validation from the pull-down menu. The following dialog box appears: (Figure 2A)

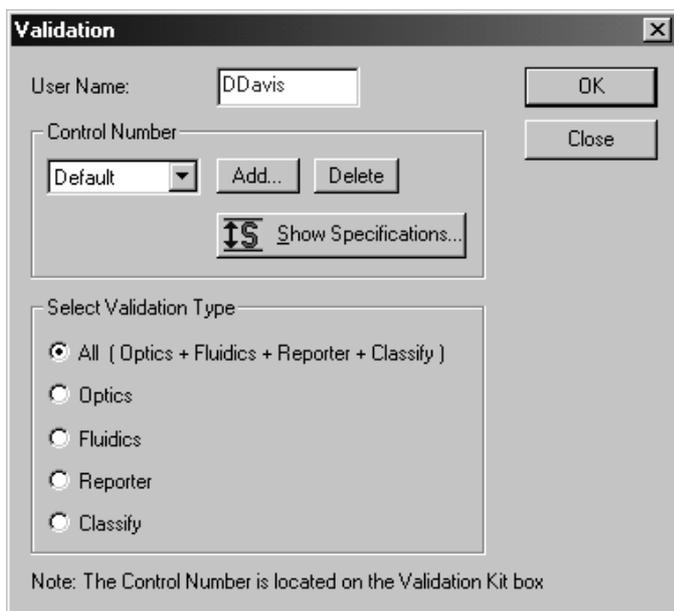


Fig. 2A. Main validation dialog.

6. Enter user name and control number. Select All. Select OK. The following dialog appears: (Figure 2B)

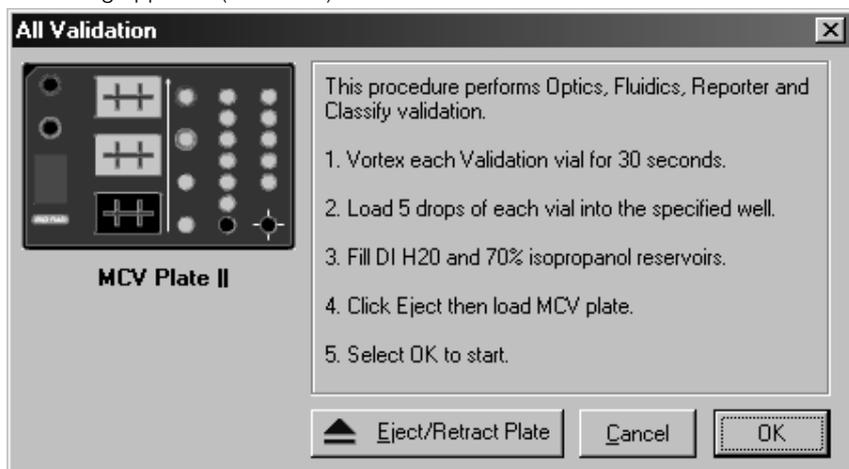


Fig. 2B.

- Place 5 drops each of bead into the respective wells on the MCV plate II (see Figure 3 below or Figure 2B). Store beads at 4°C as soon as possible after use. Protect the beads from light.

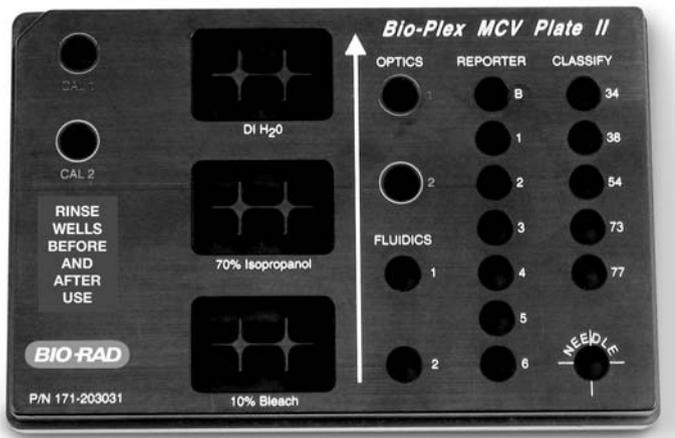


Fig. 3. MCV plate II.

- Select the Eject button in the dialog box to eject the plate holder.
- Place the MCV plate II in the microplate platform.
- Select OK to begin all validation procedures.

9.2 Validation of Optics Alignment

Procedure

- Turn on the Bio-Plex array reader, microplate platform, and computer as specified in the Bio-Plex hardware and Bio-Plex Manager user manuals.
- Perform start-up procedure as directed.
- Calibrate Bio-Plex array reader using CAL1 and CAL2 beads found in the Bio-Plex Calibration Kit according to the Bio-Plex Manager software manual.

Note: Be sure to calibrate immediately before validation. Use the High RP1 Target for CAL2 calibration.

- Remove the optics validation bead set from 4°C storage and vortex each bottle for 30 sec.
- Place 5 drops each of optics beads 1 and 2 into the respective wells on the MCV plate II.
- Store optics beads at 4°C as soon as possible after use. Protect the beads from light.
- Select Instrument from the main menu. Select Validation from the pull-down menu.

8. Enter user name and control number. Select Optics. Select OK. The following dialog appears: (Figure 4).

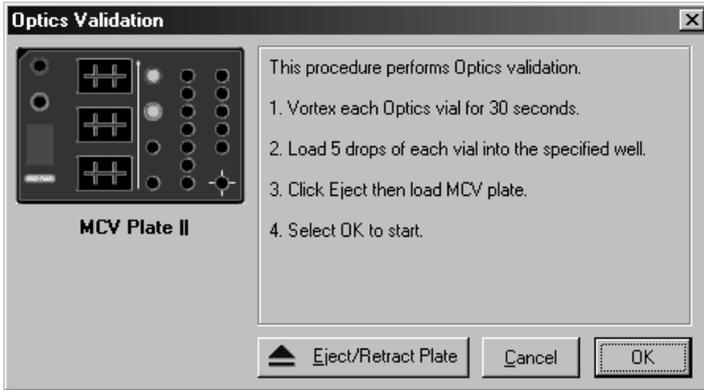


Fig. 4.

9. Select the Eject button in the dialog box to eject the plate holder.
10. Place the MCV plate II in the microplate platform.
11. Select OK to begin the optics validation procedure.
12. When the procedure has been completed, the results will be displayed in a dialog box as shown in Figure 5.
13. The results will also be logged into a validation log in Bio-Plex Manager. To access this log, select View from the main menu then Validation Log. Each type of validation is stored in a separate log for the purpose of tracking data over time. See Bio-Plex Manager 3.0 user manual for more information on using the validation log.

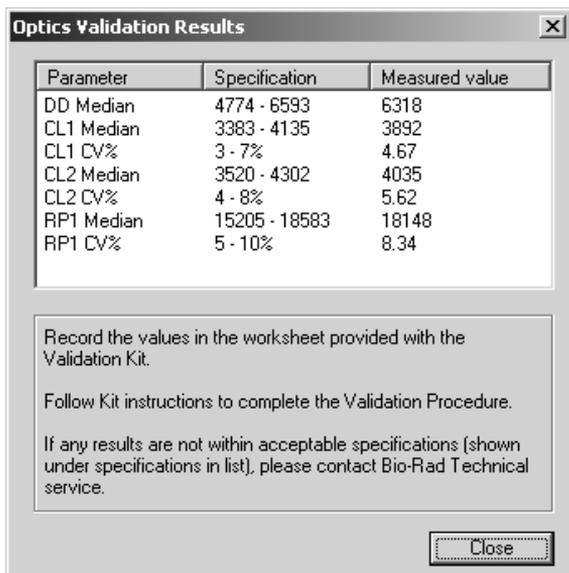


Fig. 5. Optics validation results.

14. Acceptable optics validation specifications:

Parameter	Specification
DD median	See insert
CL1 median	See insert
CL1 CV%	3–7%
CL2 median	See insert
CL2 CV%	4–8%
RP1 median	See insert
RP1 CV%	5–10%

If any values do meet specification, repeat the procedure. If values are again not within specifications, contact Bio-Rad technical support for assistance.

9.3 Validation of Fluidics Integrity

Procedure

1. If not already done, follow the procedure for start-up and calibration of the Bio-Plex system.
2. Select instrument, then validation from the main menu and select fluidics in the dialog box.
3. Enter user name and control number. Select OK, the following dialog box appears (Figure 10).

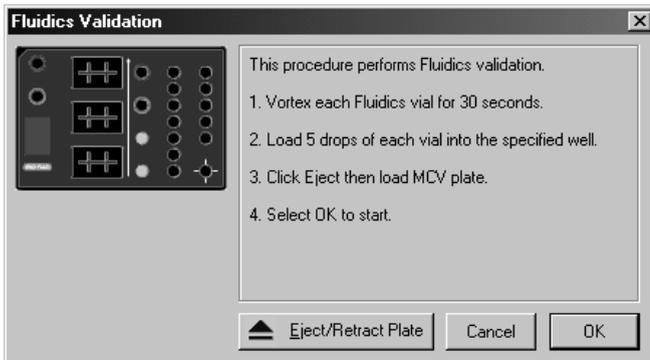


Fig. 6. Fluidics validation dialog.

4. Add 5 drops each of fluidics beads 1 and 2 to the designated wells on the MCV plate II.
5. Select the Eject button in the dialog box to eject the plate holder.
6. Place the MCV plate II in the microplate platform.
7. Select OK to begin the fluidics validation procedure.
8. When the procedure has been completed, results will appear in a dialog box as shown in Figure 11:

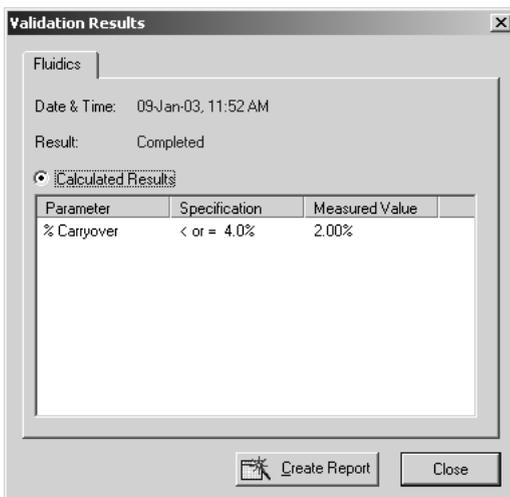


Fig. 7. Fluidics validation results.

9. Acceptable fluidics validation value.
% carryover < or = 4.0%

Note: If value is not within range, repeat the procedure. If value again is not within the specification, contact Bio-Rad technical support for assistance.

9.4 Validation of Reporter Channel Performance

Procedure

1. If not already done, follow the procedure for start-up and calibration of the Bio-Plex system.

NOTE: Be sure to calibrate immediately before validation. Use the High RP1 Target value for the CAL2 calibration.

2. Remove the reporter validation bead set from 4°C storage and vortex each bottle for 30 sec.
3. Place 5 drops of each reporter bead into the corresponding reporter well labeled as B (blank), 1, 2, 3, 4, and 5 in the MCV plate II (see Figure 2).
4. Store reporter beads at 4°C as soon as possible after use. Protect the beads from light.
5. Fill the DI H₂O reservoir with water.
6. Select Instrument from the main menu. Select Validation from the pull-down menu. A dialog will appear. Enter user name and control number. Select Reporter Validation. Select OK. The following dialog appears: (Figure 5)

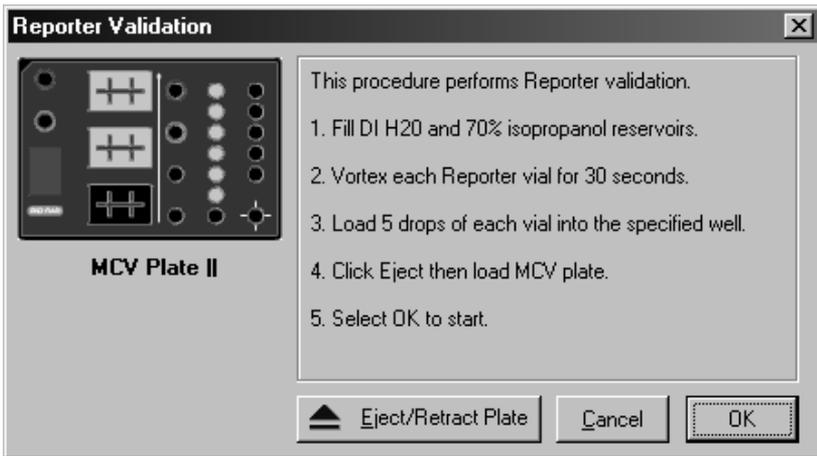


Fig. 8. Reporter validation dialog.

7. Select the Eject icon in the dialog box to eject the plate holder.
8. Place the MCV plate II in the microplate platform.
9. Select OK to start the reporter validation procedure.
10. When the procedure is completed, values will be displayed in a dialog box as shown below: (Figure 7)

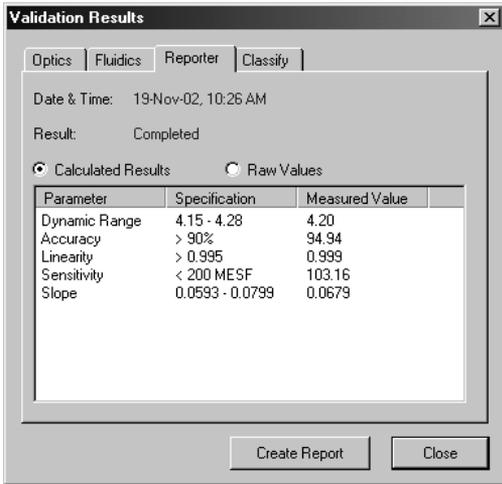


Fig. 9. Reporter validation results.

- The results will also be logged into a validation log in Bio-Plex Manager. See Bio-Plex Manager user manual for more information on using the validation log.
- Repeat procedure if values are not within specifications. If any values are again not within acceptable ranges, contact Bio-Rad technical service for assistance.
- Acceptable reporter validation specifications:

Parameter	Specification
Dynamic Range	4.15–4.28
Linearity	>0.995
Slope	0.0593–0.0799
Accuracy	>90%
Sensitivity	<200 MESF

9.5 Validation of Classify Efficiency

Procedure

- If not already done, follow the procedure for start-up and calibration of the Bio-Plex system.
- Remove the classify validation bead set from 4°C storage and vortex each bottle for 30 sec.
- Place 5 drops of each classify bead into the corresponding classify well labeled as 34, 38, 54, 73, and 77 in the MCV plate II (see Figure 2).
- Store stock vials at 4°C as soon as possible after use. Protect beads from light.

5. Select Instrument from the main menu. Select Validation from the pull-down menu.
6. Enter the User name and control number. Select classify validation. Select OK. The following dialog box appears. (Figure 8)
7. Select the Eject button to eject the plate holder.

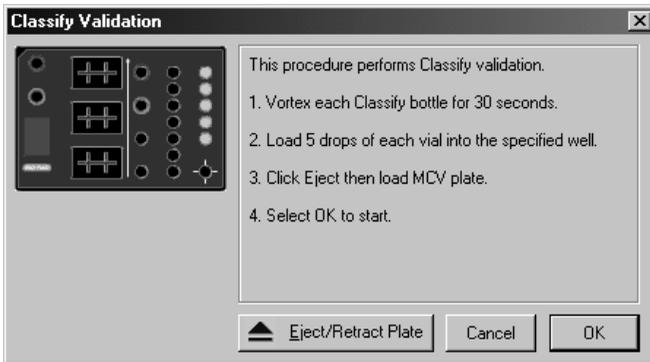


Fig. 10. Classify validation.

8. Place the MCV plate II on the microplate platform.
9. Select OK to start the classify validation procedure.
10. When the procedure is completed, values will be displayed in a dialog box as shown below (Figure 9). The classify efficiency and DD efficiency results may be accessed in this view.

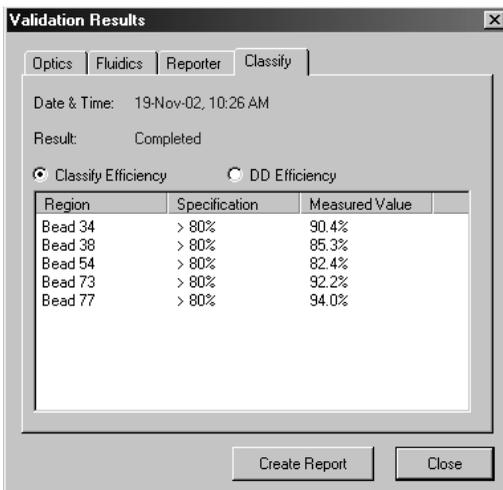


Fig. 11. Classify validation results.

11. The results will also be logged into a Validation Log in Bio-Plex Manager. To access this log, select view from the main menu then Validation Log. Each type of validation is stored in a separate log for the purpose of tracking data over time. See Bio-Plex Manager 3.0 user manual for more information on using the validation log.
12. Acceptable classify efficiency values:

Classify Bead	Specification
34	>80.0%
38	>80.0%
54	>80.0%
73	>80.0%
77	>80.0%

Note: If any values do not meet specifications, repeat the procedure. If values are again not within specifications, contact Bio-Rad technical service for assistance.

9.6 Generating a Validation Report

Procedure

The results from each validation procedure are sent to a validation log in Bio-Plex Manager 3.0. This log may be used to create individual reports as well as track multiple validation results over time. Each type of validation is logged into a separate view: optics validation, fluidics validation, reporter validation and classify validation. You may maneuver through each of the views using either the main menu items or the toolbar icons. The specifications for each control number of the validation kit are also shown in a separate window below the results. If All validation was selected, an entry matching the specific date and time will appear in each of the validation logs. All of the validation results for a specific date and time will be included in a created report. The entire log may be printed by selecting Print then Results from the main menu.

A general procedure for creating a report from the Validation log is shown below. For more detailed instructions on the use of the validation log, consult the Bio-Plex Manager 3.0 user manual.

1. Open Bio-Plex Manager 3.0 software by clicking on the application icon on the desktop.



2. Select View from the main menu then select Validation Log from the pulldown menu. The validation log will open.

3. Choose the desired validation log by using the main menu or the toolbar icons for optics, fluidics, reporter and classify validation.



4. Click on the desired entry in the validation log. The selected row will be highlighted in black.
5. Select the create report icon. A report will automatically be generated in Microsoft Excel.



6. Print the report in Excel by selecting File then Print from the main menu. Alternatively, you may use the print button in Excel.

9.7 Validation Kit Worksheet and Report Form Examples

The following is a sample validation report from Bio-Plex Manager. Note that the values included here are for demonstration purposes only. Consult your product insert for values specific to your product control number..

Bio-Plex Suspension Array System Validation Report

Test Performed by: DanoDate/Time: 01-13-2003 12:20Validation Kit Control #: 90610Reader Serial #: 1x10099092008RP1 Target Value: 17677RP1 PMT Voltage: 808.79**I. Optics Validation**Result: Completed

Parameter	Specification	Measured Value	Pass/Fail
DD Median	4774 - 6593	5799	Pass
CL1 Median	3383 - 4135	3732	Pass
CL1 CV%	3.00 - 7.00	6%	Pass
CL2 Median	3520 - 4302	3892	Pass
CL2 CV%	4.00 - 8.00	7%	Pass
RP1 Median	15205 - 18583	17332	Pass
RP1 CV%	5.00 - 10.00	8%	Pass

II. Fluidics ValidationResult: Completed

Parameter	Specification	Measured Value	Pass/Fail
% Carryover	< or = 3.5%	2%	Pass

III. Reporter ValidationResult: Completed**A. Calculated Values**

Parameter	Specification	Measured Value	Pass/Fail
Dynamic Range	4.15 - 4.28	4.1936	Pass
Linearity	> 0.995	1.000	Pass
Slope	0.0593 - 0.0799	0.0664	Pass
Accuracy	> 90.00%	98%	Pass
Sensitivity	< 200 MESF	120	Pass

B. Raw Values

Bead	Median FI
Blank	8
1	19
2	182
3	2516
4	7006
5	15624

IV. Classify ValidationResult: Completed**A. Classify Efficiency**

Classify Bead	Specification	Measured Value	Pass/Fail
Bead 34	> 80.0%	99%	Pass
Bead 38	> 80.0%	97%	Pass
Bead 54	> 80.0%	96%	Pass
Bead 73	> 80.0%	97%	Pass
Bead 77	> 80.0%	97%	Pass

B. DD Efficiency

Parameter	Specification	Measured Value	Pass/Fail
DD Efficiency	> or = 75.0%	91%	Pass

Comments: _____

Reviewed by: _____

Date: _____

Section 10

Procedure for Bio-Plex Manager 2.0 and MCV Plate

10.1 Validation of Optics Alignment

Procedure

1. Turn on the Bio-Plex array reader, microplate platform, and computer as specified in the Bio-Plex hardware and Bio-Plex Manager user manuals.
Note: Be sure to calibrate immediately before validation. Use the High RP1 Target value for CAL2 calibration.
2. Perform start-up procedure as directed.
3. Calibrate Bio-Plex array reader using CAL1 and CAL2 beads found in the Bio-Plex calibration kit according to the Bio-Plex Manager software manual.
4. Remove the optics validation bead set from 4°C storage and vortex each bottle for 30 sec.
5. Place 5 drops of optics bead 1 into the first of the 3 optics wells labeled with "1" on the MCV plate (see Figure 2 below). Leave the 2 remaining wells labeled with "1" empty.

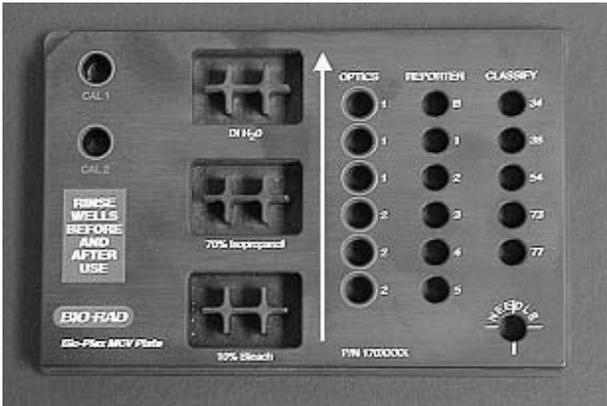


Fig. 12. MCV plate.

6. Place 5 drops of optics bead 2 into the first of the 3 optics wells labeled with 2 on the MCV plate. Leave the 2 remaining wells labeled with "2" empty.
7. Store optics beads at 4°C as soon as possible after use. Protect the beads from light.

8. Select Instrument from the main menu. Select Validation from the pull-down menu. Select Optics from the pop-up menu. The following dialog box appears:

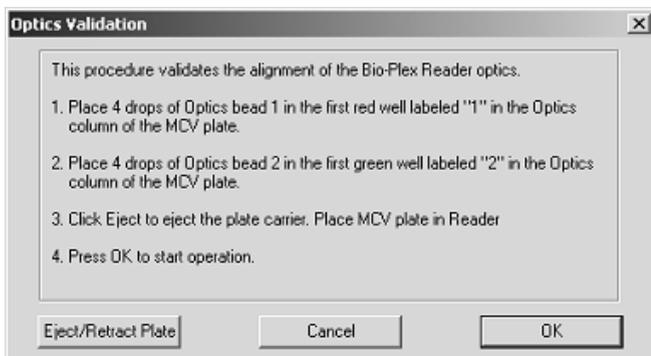


Fig. 13. Optics validation dialog.

9. Select the Eject button in the dialog box to eject the plate holder.
10. Place the MCV plate in the Bio-Plex microplate platform.
11. Select OK to begin the optics assessment procedure.
12. When the procedure has been completed values will be displayed in a dialog box as shown on the next page.
13. Record the values for each optics validation parameter in the validation kit worksheet provided with the validation kit.

Parameter	Specification	Measured value
DD Median	4774 - 6593	6318
CL1 Median	3383 - 4135	3892
CL1 CV%	3 - 7%	4.67
CL2 Median	3520 - 4302	4035
CL2 CV%	4 - 8%	5.62
RP1 Median	15205 - 18583	18148
RP1 CV%	5 - 10%	8.34

Record the values in the worksheet provided with the Validation Kit.

Follow Kit instructions to complete the Validation Procedure.

If any results are not within acceptable specifications (shown under specifications in list), please contact Bio-Rad Technical service.

Close

Fig. 14. Optics validation results.

14. Acceptable optics validation specifications:

Parameter	Specification
DD median	See insert
CL1 median	See insert
CL1 CV%	3–7%
CL2 median	See insert
CL2 CV%	4–8%
RP1 median	See insert
RP1 CV%	5–10%

If any results are not within the acceptable specifications, please contact Bio-Rad technical service.

If any values do meet specification, repeat the procedure. If values are again not within specifications, contact Bio-Rad technical service for assistance.

10.2 Validation of Reporter Channel Performance

Procedure

1. If not already done, follow the procedure for start-up and calibration of the Bio-Plex system. Note: Be sure to calibrate immediately before validation. Use the High RP1 Target value for CAL2 calibration.
2. Remove the reporter validation bead set from 4°C storage and vortex each bottle for 30 sec.

3. Place 5 drops of each reporter bead into the corresponding reporter well labeled as B (blank), 1, 2, 3, 4, and 5 in the MCV plate (see Figure 2).
4. Store reporter beads at 4°C as soon as possible after use. Protect the beads from light.
5. Fill the DI H₂O reservoir with water.
6. Select Instrument from the main menu. Select Validation from the pull-down menu. A dialog box will appear. Select Reporter from the pop-up menu. The following dialog box appears:



Fig. 15. Reporter validation dialog.

7. Select the Eject icon in the dialog box to eject the plate holder.
8. Place the MCV plate in the Bio-Plex microplate platform.
9. Select OK to start the reporter validation procedure.
10. When the procedure is completed, values will be displayed in a dialog box as shown below:

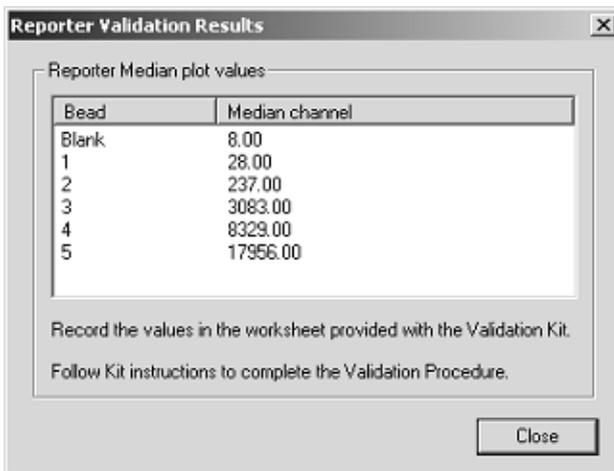


Fig. 16. Reporter validation results.

11. Record the median channel values in a copy of the worksheet provided with the validation kit.
12. Repeat procedure if values are not within specifications. If any values are again not within acceptable ranges, contact Bio-Rad technical service for assistance.
13. Acceptable reporter validation specifications:

Parameter	Specification
Dynamic Range	4.15–4.28
Linearity	>0.995
Slope	0.0593–0.0799
Accuracy	>90%
Sensitivity	<200 MESF

10.3 Validation of Classify Efficiency

Procedure

1. If not already done, follow the procedure for start-up and calibration of the Bio-Plex system. Note: Be sure to calibrate immediately before validation. Use the High RP1 Target value for CAL2 calibration.
2. Remove the classify validation bead set from 4°C storage and vortex each bottle for 30 sec.
3. Place 5 drops of each classify bead into the corresponding classify well labeled as 34, 38, 54, 73, and 77 in the MCV plate (see Figure 2).
4. Store stock vials at 4°C as soon as possible after use. Protect beads from light.
5. Select Instrument from the main menu. Select Validation from the pull-down menu. Select Classify from the pop-up menu. The following dialog box appears:

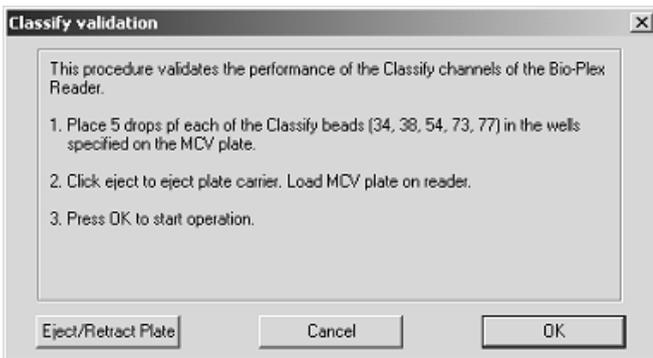


Fig. 17. Classify validation dialog.

6. Select the Eject button to eject the plate holder.

7. Place the MCV plate on the Bio-Plex microplate platform.
8. Select OK to start the classify validation procedure.
9. When the procedure is completed, values will be displayed in a dialog box as shown below:

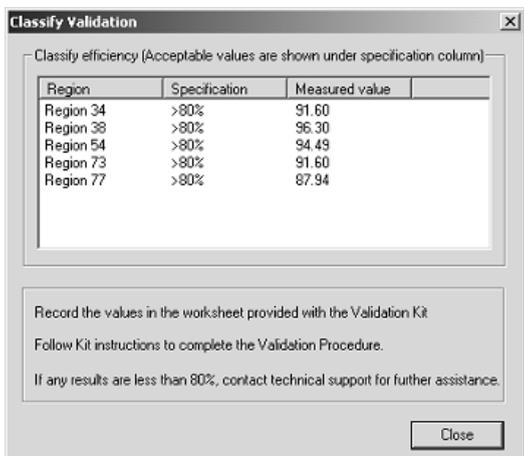


Fig. 18. Classify Validation dialog.

10. Record the classify efficiency values in a copy of the validation kit worksheet provided in this manual.
11. Acceptable classify efficiency values:

Classify Bead	Specification
34	>80.0%
38	>80.0%
54	>80.0%
73	>80.0%
77	>80.0%

Note: If any values do not meet specifications, repeat the procedure. If values are again not within specifications, contact Bio-Rad technical service for assistance.

10.4 Validation of Fluidics Integrity

Procedure

1. If not already done, follow the procedure for start-up and calibration of the Bio-Plex system. Note: Be sure to calibrate immediately before validation. Use the High RP1 Target value for CAL2 calibration.
2. Remove the calibration bead set from 4°C storage and vortex each bottle for 30 sec.

2. Select the Unclog icon from the toolbar.
3. The following dialog box appears:

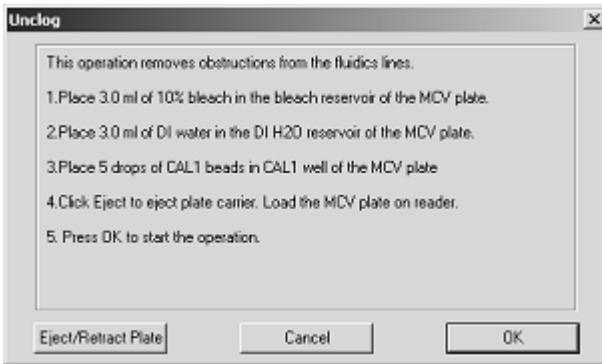


Fig. 19. Unclog dialog.

4. Add 5 drops of CAL1 beads to the CAL1 well on the MCV plate.
5. Fill the DI H₂O and 70% isopropanol reservoirs.
6. Select the Eject button in the dialog box to eject the plate holder.
7. Place the MCV plate in the Bio-Plex microplate platform.
8. Select OK to begin the validation of fluidics integrity procedure.
9. If the unclog procedure was successful, the following message will appear:



10. Check "yes" in Section IV of the validation kit worksheet.
11. If the procedure is unsuccessful, the following message will appear: If this is the case, repeat steps 4–11.



12. If the procedure is again unsuccessful, contact Bio-Rad technical support for assistance.

10.5 Generating a Validation Report

Procedure

1. Insert the Bio-Plex Manager CD into your computer's CD drive.
2. Open Microsoft Excel®.
3. Select Open and using your browser choose Bio-Plex validation report from the Bio-Plex CD.
4. Click on the Worksheet tab.
5. Enter the values from the worksheet used during the validation procedure. A series of calculations will be automatically performed.
6. Click on the Report Tab to view the Validation report.
7. Values out of specifications will be shown in red.
8. Select File, then Print to print the report.
9. Select File then Save.
10. Enter the desired file name and location, then select OK to save the file.
11. If any values are not within acceptable specifications, repeat that specific validation procedure.
12. If values are not within acceptable specifications after repeating a second time, contact Bio-Rad technical support for assistance.

10.6 Validation Kit Worksheet and Report Form Examples

The following is a sample validation kit worksheet used to record values obtained during the various validation procedures. A full-size version suitable for photocopying is included with the kit. Also included is an example of the validation kit report output from Bio-Plex Manager (see Section 13). Note that the values included here are for demonstration purposes only. Consult your product insert for values specific to your product control number.



Protein Array Reader Validation Kit Worksheet

Performed by: _____

I. Optical Validation

Parameter	Specification	Measured Value
DD median	4774-6593	
CL1 median	3383-4135	
CL1 CV%	3-7%	
CL2 median	3520-4302	
CL2 CV%	4-8%	
RP1 median	15205-18583	
RP1 CV%	5-10%	

II. Reporter Validation

Bead	Median Channel
B(Blank)	
1	
2	
3	
4	
5	

III. Classify Validation

Region	Specification	Measured Value
Region 34	>80%	
Region 38	>80%	
Region 54	>80%	
Region 73	>80%	
Region 77	>80%	

IV. Validation of Fluidics Integrity

Fluidics Integrity Validated? (Place "X" in appropriate box)

YES	NO

To Generate Report:

1. Open Excel and open the ValidationReport.xls file.
2. Click on 'Worksheet' tab and enter values from this worksheet.
3. Click on Report and select 'File' then 'Print' to print report.

4106178 Rev A



Protein Array Reader Validation Report

Performed by: _____
Serial Number: _____
Kit Lot number: _____

I. Optics Validation

Parameter	Specification	Measured Value
DD median	4774-6593	6150
CL1 median	3383-4135	3661
CL1 CV%	3-7%	5.35
CL2 median	3520-4302	3854
CL2 CV%	4-8%	6.43
RP1 median	15205-18583	17201
RP1 CV%	5-10%	6.51

II. Reporter Validation

Parameter	Specification	Measured Value
Dynamic Range	4.15-4.28	4.23
Linearity	>0.995	1
Slope	.0593-.0799	0.723
Accuracy	>90%	95.37%
Sensitivity	<200 MESF	124.41

III. Classify Validation

Region	Specification	Measured Value
Region 34	>80%	96.63%
Region 38	>80%	95.94%
Region 54	>80%	93.88%
Region 73	>80%	95.14%
Region 77	>80%	95.36%

Note: Values in **red** indicate parameters not within specifications. If values are not within acceptable specifications, please repeat procedure. If values are still not within specifications, contact Bio-Rad technical service for assistance.

See Bio-Plex validation kit manual for specific definitions of each parameter and the impact on Bio-Plex assay performance.



IV. Validation of Fluidics Integrity

Fluidics integrity validated?

Note: Values in **red** indicate parameters not within specifications. If values are not within acceptable specifications, repeat procedure. If values are still not within specifications, contact Bio-Rad technical service for assistance.

See Bio-Plex validation kit manual for specific definitions of each parameter and the impact on Bio-Plex assay performance.

Comments: _____

Sample

Reviewed by: _____ Date: _____

Section 11

Troubleshooting Guide

Problem	Cause	Solution
Optics validation procedure shows value outside of acceptable range	Problem with the optical component of the array reader	Repeat the procedure. If value is still out of range, contact Bio-Rad technical support.
Reporter validation procedure shows value outside of acceptable range	Problem with the optical component of the array reader	Repeat the procedure. If value is still out of range, contact Bio-Rad technical support.
Classify validation procedure shows value outside of acceptable range	Problem with the calibration or optical component of the array reader	Repeat the procedure. If values are still out of range, contact Bio-Rad technical support.
Fluidics validation procedure shows value outside of acceptable range	Problem with fluidics lines, valves or sample needle of array reader.	Repeat procedure. If value is still out of range, contact Bio-Rad technical support.

Section 12

Ordering Information

Catalog #	Description
171-203000	Bio-Plex Validation Kit 3.0, includes optics validation, fluidics validation, reporter validation, and classify validation bead sets for approximately 50 validation routines
171-203060	Bio-Plex Calibration Kit, includes CAL1 and CAL2 calibration beads for approximately 50 daily calibration routines
171-203031	Bio-Plex MCV Plate II for use with Bio-Plex Manager 3.0 and Validation kit 3.0
171-203030	Bio-Plex MCV Plate for use with Bio-Plex Manager 2.0 and Validation kit 3.0

Section 13

Reference

Alder, Henry: Introduction to Probability and Statistics. Alder HL and Roessler EB (eds) W.H. Freeman, San Francisco, p118 (1968).

By purchasing this kit, which contains fluorescent labeled microsphere beads authorized by Luminex, you, the customer, acquire the rights under Luminex's patent rights* to use certain portions of this kit, including without limitation the microsphere beads contained herein, only with Luminex's laser-based fluorescent analytical test instrumentation known under the name of Luminex 100, for example as marketed by Bio-Rad Laboratories, Inc., in the Bio-Plex system.

* Including, but not limited to US patent 5,981,180; 6,046,807; 6,057,107

Certain Bio-Plex validation kit components are licensed under US patent 5,723,218