



AssayCheX™
Process Control Panel
For Immunoassays
Catalog# PCP01

www.RadixBioSolutions.com
512.869.8000

AssayCheX™ Process Control Panel For Immunoassays Catalog# PCP01

AssayCheX 4-plex Process Control Panel 01 for immunoassays

Read entire contents before use.
For Research Use Only.

Document Number 00074R Rev. A (Effective 5.7.2009)

TABLE OF CONTENTS

Contents	Page
Application	2
Compatible Matrices	2
Storage	2
Background	2
Directions for Use	5
Appendix	
Establishing Threshold Metrics	6
Plate Failure Threshold	6
Initial Acceptance Ranges	6
Obtaining Kit-specific Threshold Values	8
Alternate Acceptance Range Criteria	11

MANUFACTURED AND DISTRIBUTED BY:

Radix BioSolutions, Ltd.
111 W Cooperative Way, Suite 120
Georgetown, TX, USA 78626
Phone: 512.869.8000
Fax: 512.868.9040
Email: info@RadixBioSolutions.com

APPLICATION

This product may be used in conjunction with any immunoassay for the Luminex® xMAP® platform. *This product is for research use only.*

COMPATIBLE MATRICES

Buffer, plasma, serum, milk, or culture supernatants.

STORAGE

Store in the dark at 2-8°C. DO NOT FREEZE.

BACKGROUND

The AssayCheX process control panel is designed to provide assurance of the validity of each sample's results for immunoassays performed using Luminex xMAP technology. Whereas Luminex provides calibrators and controls to ensure a properly functioning instrument, this 4-plex panel utilizes two independent acceptance metrics to ensure that each step of the immunoassay is performed correctly and that the instrumentation is operating properly at

the time of data acquisition. Inclusion of these controls in every well enables a user to invalidate an entire plate in the event of an instrument failure or a kit failure or to ignore individual sample data in the event of an intermittent instrument error or sporadic operator error. Table 1 displays how the AssayCheX panel monitors different steps of an immunoassay.

Table 1. AssayCheX Panel Components

Microsphere Region	Name	Significance
97	Instrument Control	Monitors the Luminex analyzer reporter fluorescence measurement
98	Fluorescent Reporter Control	Monitors the addition of the avidin-conjugated fluorescent reporter reagent to the well
99	Biotin Detector Control	Monitors the addition of the biotin-labeled detector antibody reagent to the well
100	Negative or Non-specific Control	Monitors the non-specific binding (if any) due to the sample matrix

The first acceptance metric is composed of a set of threshold values for the AssayCheX panel. These threshold values are designed to function as an entire plate diagnostic parameter to identify any error due to catastrophic instrument or reagent failure. Such an error can result in false negative values that are overlooked in the absence of AssayCheX.

The second acceptance metric consists of a range of acceptable fluorescence values for each AssayCheX microsphere region to ensure that each well of an assay plate has been processed properly and that the instrument has operated properly at the time of data acquisition. Acceptance criteria values are presented as a range of high and low acceptance values to accommodate normal assay variance.

Sample results in which the AssayCheX results fall within the acceptance range are considered valid. Whereas, sample results in which the AssayCheX results are outside the acceptance range are identified as potential invalid results. The pattern of AssayCheX values may also be used as a troubleshooting guide to indicate which specific process of the assay has failed.

DIRECTIONS FOR USE

Tip: Mix microspheres well before use by vortexing or pipetting up and down.

1. Add the AssayCheX process control panel microspheres to your assay's 1X microsphere mixture immediately prior to adding the microspheres to the wells. Add one (1) μL of AssayCheX microspheres to the 1X microsphere mixture for each well to be tested (for examples see Table 2).

****CAUTION:** Add AssayCheX microspheres *only* to the volume of analyte-specific microspheres that will be used immediately**

Table 2. AssayCheX Addition Examples

Wells To Be Tested	Volume of AssayCheX Microspheres Added
25	25 μL
100	100 μL

2. Perform assay procedure as per the assay kit protocol.
3. For data acquisition, select the AssayCheX microsphere regions in addition to the kit-specific regions in an xMAP data acquisition/analysis software package.
4. To confirm the validity of each sample result during data analysis, plate threshold values and acceptance criteria ranges must be determined.

APPENDIX

Establishing Threshold Metrics

To properly utilize the AssayCheX panel for confirming the validity of assay results, acceptance metrics must be established. We provide initial threshold and acceptance range values as guides to initiate testing. However, for optimal results, we recommend that the end user establishes more precise values based on the performance of their specific kit and samples.

Plate Failure Threshold

Table 3 provides recommended threshold values for each AssayCheX microsphere region. AssayCheX results that are acceptable based on the threshold values confirm a properly operating instrument and a functional assay kit at the time of data acquisition. For microsphere region 100, an MFI value below the threshold value indicates an acceptable result; for microsphere regions 97, 98, and 99, values above the threshold indicate an acceptable result.

Initial Acceptance Ranges

Table 4 provides recommended acceptance ranges for each AssayCheX microsphere regions. The ranges can be established as discrete fluorescence values or based on statistical measures determined from the mean fluorescence values generated from all of the unknown samples analyzed for each AssayCheX microsphere region.

Table 3. Plate Failure Threshold Values.

Microsphere Region	Initial Recommended Threshold Value	Results Acceptance: Above or Below Threshold Value
97	300	Above
98	300	Above
99	200	Above
100	75	Below

Table 4. Recommended Acceptance Ranges.

Microsphere Region	Acceptance Range
97	\pm 30% of average MFI from unknown sample values only
98	\pm 30% of average MFI from unknown sample values only
99	\pm 30% of average MFI from unknown sample values only
100	+ 100%/ -50% of average MFI from unknown sample values only

We recommend that the end user establishes more precise values based on the performance of their specific kit and samples.

The following provides guidance on best practices for determining plate threshold values and acceptance criteria ranges values.

Obtaining Kit-specific Threshold Values

If a significant number of samples are to be analyzed by the same kit multiplex over a period of time, then for optimal results it is recommended that the end user determine kit-specific threshold MFI values for the AssayCheX panel. The description below outlines a procedure for determining the threshold values for each AssayCheX microsphere region based on the specific assay kit in use.

- 1) Dilute 20 μL of the AssayCheX microspheres in 480 μL of assay kit buffer. As a surrogate sample utilize the sample matrix to be tested and, if necessary, dilute with the appropriate buffer as outlined in the kit protocol.
- 2) Add the microspheres and surrogate sample to a plate as detailed below and incubate as prescribed in the kit protocol.
- 3) Proceed with the kit protocol with the following exceptions:
 - a) Treatment Group 1. For wells 1-4 add reagent diluent/assay buffer

in the absence of any biotin conjugated detection antibody or Streptavidin-Phycoerythrin.

- b) Treatment Group 2. For wells 4-8 add reagent diluent/assay buffer in the absence of any biotin conjugated detection antibody; however, do add the appropriate amount of Streptavidin-R-Phycoerythrin as indicated in the kit protocol.
- c) Treatment Group 3. For wells 9-12 add all reagents as outlined in the kit protocol.
- 4) For data acquisition, establish a new acquisition protocol with chosen xMAP acquisition software and select microsphere regions 97, 98, 99 and 100 and collect 100 events per microsphere region.
- 5) Export the acquired data to a spreadsheet and calculate average MFI values for each of the three treatment conditions for each of the four microsphere regions 97, 98, 99, and 100.

Treatment Group 1 is used to determine the base value for calculating the Threshold Value for acceptance of data based on AssayCheX microsphere region 97. Treatment Group 3 is used to determine the base values for calculating the Threshold Values for acceptance of data based on AssayCheX microsphere regions 98, 99, and 100. The blacked out regions of Table 5 including all of Treatment Group 2 provide redundant data or negative control data to be used only as a guide to ensure that the assay kit provides reagents capable of discerning positive assay results from negative assay results.

For acceptable data, the AssayCheX values obtained during sample testing for microsphere regions 97, 98, and 99 must be greater than the Threshold Values, and for microsphere region 100 the values must be below the Threshold Value.

Table 5. Kit-Specific Threshold Value Calculator.

Microsphere Region	Treatment Group			Threshold Calculation (multiply Average MFI by)	Threshold Value
	1	2	3		
97				0.25	
98				0.25	
99				0.25	
100				2	

In Table 5 insert the average MFI values for each AssayCheX microsphere region into the appropriate treatment group, multiply by the Threshold Calculation Value to obtain the kit-specific Threshold Value for determination of acceptable assay plate and instrument performance.

Alternate Acceptance Range Criteria

The initial acceptance range criteria is based on determining the average MFI values for each AssayCheX microsphere region using data from unknown sample data only. AssayCheX values from standard curve results are not included in the calculations due to differences in MFI values for AssayCheX microspheres based on sample/standard matrix effects. The initial acceptance range values are based on calculations of a percentage of the average MFI values above and below the average. Alternative methods for determining acceptance ranges are to establish set MFI values regardless of the average MFI or to develop a range based on the standard deviation of results for each AssayCheX microsphere region average result, generally ± 2 or 3 SD of the mean (average). The initial criteria are based on a percentage of the average because in general, the distribution of MFI values for the AssayCheX panel is very tight. Therefore, developing a range based on the standard deviation can result in very tight acceptance ranges and produce an unreasonable number of AssayCheX failures which bear little relationship to the assay kit performance for the failed results.



Luminex[®], xMAP[®] and xMAP[®] medallion are registered trademarks of Luminex Corporation. AssayCheX[™] is a trademark of Radix BioSolutions, Ltd.

Stop Guessing!

The AssayCheX process control panel provides excellent guidance for confirming the quality and validity of each sample result. It allows the end user to be confident that their multiplexed immunoassay data has been properly generated with Luminex xMAP technology.

www.RadixBioSolutions.com
111 W Cooperative Way, Suite 120
Georgetown, TX, USA 78626
Phone: 512.869.8000
Fax: 512.868.9040

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex Corporation ("Luminex"), you, the customer, acquire the right under Luminex's patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex's laser-based fluorescent analytical test instrumentation marketed under the name of Luminex 100™ IS, 200™, or HTS.