Glossary

Accuracy The closeness of a measured value to the actual value, or the difference between expected and measured values. Using the reporter validation kit, accuracy is defined as the percent difference between the measured regression curve and the expected or actual data points.

Aggregated microspheres Microspheres that have become associated. Two microspheres that have associated are called a doublet.

Analyte The substance being measured, identified, quantitated, or otherwise analyzed in an experiment. In the Bio-Plex system, analytes are molecules that are being quantitated.

Antibody Immunological protein capable of binding a unique antigen via a specific binding site, or epitope. Used in immunological assays to quantitate the amount of a specific analyte present.

Assay The bead and reagent mixture that, when combined with sample, is loaded into microplate wells and is read by the Bio-Plex array reader.

Background Fluorescence intensity measured by the reporter channel that is due to nonspecific binding of a fluorophore and the electronic noise of the array reader. If you include blank wells in your microplates, you can calculate the average background intensity and subtract it from the intensity readings of your standards and unknowns.

Bead A microsphere.

Calibration A process that uses microspheres (calibrators) with a stable fluorescence intensity to adjust the gain settings in the Bio-Plex array reader's detectors for optimal and consistent microsphere classifications and reporter readings, over time and across different array readers.

Calibrators Microspheres with stable fluorescence intensities used to standardize the signal output of the Bio-Plex array reader.

Capture antibody An antibody used in sandwich immunoassays that binds, or "captures", a specific analyte for quantitation.

Carboxylated microspheres Microspheres with chemically modified surfaces such that they are covered with carboxyl functional groups (COOH) that allow covalent biomolecular attachment.

Classification channels Fluorescent detection channels of the Bio-Plex array reader that detect the fluorescence intensities of two dyes embedded in the Bio-Plex microspheres. The ratio of the two dyes identifies each bead as a unique analyte within a sample.

Classify A validation procedure for measuring the validation ability of the Bio-Plex array reader to efficiently classify assay beads into specified regions.

Clumping The aggregation of two or more microspheres.

Conjugated microspheres A microsphere set that has been chemically modified so that its surfaces are covered with molecules that bind with the target analytes in an assay system.

Coupled microsphere See conjugated microsphere.

Cytokine An immunological protein that can signal a response in the immune system.

Detection antibody An antibody used in capture sandwich immunoassays that binds a captured analyte to a fluorophore, allowing quantitation of the protein. Detection antibodies may be biotinylated, which allows addition of a streptavidin-bound fluorophore, or the detection antibody may be directly labeled with a fluorophore.

Doublet Two microspheres that have associated. The resulting signal of the associated microspheres is twice that of a single (singlet) microsphere.

Doublet discriminator A channel that measures the amount of light scattering from particles that flow past the red laser. Light scattering is directly proportional to particle size, and the channel is designed to identify particles that are smaller or larger than a single microsphere, including microspheres that are clumped or aggregated.

Dynamic range The calculated number of decades covered by the log amplifier and the reporter channel scale of the Bio-Plex array reader. Range is calculated using the reporter validation kit.

Emission spectrum The wavelength range emitted by an excited fluorophore when its electrons fall from a higher to a lower energy state. Expressed in nanometers (nm).

Ex/Em A common way of reporting a molecule's excitation and emission wavelengths (for example, Ex/Em = 488/520 nm).

Excitation spectrum The wavelength range that excites a molecule's electrons to a higher energy state. Expressed in nanometers (nm).

External standard A standard that has been imported from a different file.

Fit probability A statistical measure of goodness of fit that incorporates the residual SSE. A fit probability of 1 indicates a perfect fit, while a fit probability of 0 indicates no fit.

Fluidics validation The process of verifying the well-to-well carryover of beads in the Bio-Plex array reader.

Fluorescence The emission of light that occurs when the electrons of a fluorophore drop to a lower energy state.

Fluorochrome Same as fluorophore.

Fluorophore A fluorescent molecule.

Gate An electronic method of discriminating or eliminating aggregated microspheres in the analysis of an assay. The Doublet Discrimination gate separates singlet beads from aggregated beads.

Group A group of wells defined on a plate. You can calculate the ratio of the fluorescent intensities of the group's member wells to the fluorescence intensity of a reference well in the group.

Immunofluorescence A technique that uses a covalently linked fluorophore-antibody complex to detect or quantify a particular antigen.

Kinase An enzyme that functions to phosphorylate specific proteins. Kinases are responsible for the activation of a variety of proteins through the process of phosphorylation.

Laser A highly purified source of light used to excite fluorophore electrons. An acronym for light amplification by stimulated emission of radiation.

Linearity A measurement of the coefficient of determination, or r^2 value, of a set of standard beads. An r^2 value of 0.995 or greater is an acceptable linearity value using the Bio-Plex validation kit.

Logistic regression A regression model for binary (dichotomous) outcomes. The data are assumed to follow binomial distributions with probabilities that depend on the independent variables.

Median fluorescence intensity A relative measurement of fluorescence intensity based on the median, a robust statistical measure.

Microparticle A solid substance with a diameter in the micrometer range. Often used as a synonym for a microsphere.

Microsphere set A set of Bio-Rad multianalyte microspheres containing a unique mixture of two distinct fluorophores to distinguish them from other multianalyte microspheres.

Microspheres Latex spheres with a diameter in the micrometer range. Also called beads.

Multianalyte or multiplex An analysis of several assays or tests performed simultaneously in the same reaction container.

Operational qualification The process of determining that an instrument is fit for its intended use. The Bio-Plex validation kit is a tool for operational qualification.

Optics validation The process for verifying the alignment of the optics assembly of the Bio-Plex array reader.

Outlier A well or replicate group of wells whose measured value is outside the normal range. You can exclude outliers from statistical calculations.

Panel A group of related bead sets. For example, the human cytokine panel includes bead sets for human IL-2, human IL-4, human IFN-γ, and other human cytokines. You can select multiple bead sets from the same panel or sets from different panels in the same multiplex assay.

PE See phycoerythrin.

Phosphoprotein An enzyme that functions to phosphorylate specific proteins. Phosphoproteins are responsible for the activation of a variety of proteins through the process of phosphorylation.

Photobleaching A chemical reaction caused by exposure to light, in which a fluorophore is converted into a differently fluorescent or nonfluorescent compound.

Photomultiplier tube (PMT) A light detector typically used in fluorescence detection systems, designed to convert a fluorescent signal into an electronic signal that can be quantitated.

Phycoerythrin (PE) The fluorophore used as the reporter molecule in Bio-Plex assays. Excitation = 546 nm, emission = 575 nm.

PMT See photomultiplier tube.

Precision A measure of the reproducibility of replicate readings, usually represented by the coefficient of variation (CV%).

Protocol file (*.pbx) A file containing the settings of a reading, including the microplate wells to read, the analytes to detect, sample size, etc. To perform a reading, you open a Protocol file, select the settings, and then run the Protocol. You can then save the Protocol settings and reuse or modify them. After a reading, a Results file is created containing the settings from the Protocol as well as the data results. The raw data from the most recent reading is also stored in the Protocol file.

Quality control Procedures and guidelines that determine conformity to requirements.

Recovery percentage A mechanism for assessing the fit of the standard curve to the actual standards. For each analyte standard, an observed concentration is back-calculated from the standard curve and the fluorescence intensity. This is divided by the expected concentration and multiplied by 100 to give the recovery percentage.

Recovery range The range of acceptable recovery percentages (see above). For example, a recovery range of 80–120% means that the observed concentration of a standard should be within 80–120% of the expected concentration. Concentrations of standards and unknowns that fall outside this range are flagged as unreliable.

Reference A well within a group of wells defined on a plate that is used as a reference. You can calculate the ratio of the fluorescence intensity of the reference well to the fluorescence intensities of the other wells in the group.

Region The region of a fluorescent color map used to identify a particular bead set. Each bead set is embedded with specific quantities of two fluorescent dyes; the combination of these fluorophores, as detected by the Bio-Plex array reader, places the bead set within a unique region on the color map, thereby identifying the set and its associated analyte.

Reporter A fluorescent molecule that is incorporated into an assay in such a way that the fluorescence intensity is directly proportional to the amount of analyte in an assay.

Reporter channel The channel of the Bio-Plex array reader that detects the fluorescent signal of the reporter molecule (phycoerythrin).

Reporter system Any combination of molecules in solution that serves to quantitate the analytes in a particular assay.

Reporter validation A standard method for verifying the performance of the reporter channel of the Bio-Plex array reader. The five primary parameters that verify accurate performance of the reporter channel include linearity, dynamic range, sensitivity, accuracy, and slope of the response.

Residual variance A statistical measure of goodness of fit. This is the weighted sum of the squared errors between the observed response and the response predicted by the fitted curve (residual SSE), divided by the number of degrees of freedom. A small residual variance indicates a good curve fit.

Results file (*.rbx) A file containing the results of a reading, including the raw data, the settings information from the Protocol, and tools for analyzing and exporting the data (tables, a standard curve, export functions, etc.). Each reading generates a new Results file.

Sample A solution of analyte standards or unknowns that, when added to an assay, will bind to the microspheres in that assay and be identified and quantitated by the array reader.

Sampling error A measure of the degree to which a sample differs from the entire population.

Sandwich immunoassay Immunoassay for the quantitation of a specific analyte through the use of monoclonal antibodies (Ab). A sandwich is formed when an analyte of interest binds to a capture Ab, and then a second antibody (detection Ab) binds to a different epitope of the protein.

Sensitivity The lowest detectable signal above instrument noise. Noise may be attributed to the lasers, the detectors, and the amplification electronics of the array reader.

Signal The detectable measurement unit of a reporter molecule.

Signal-to-noise ratio The ratio of a specific assay signal to the underlying noise in the assay. Signal to noise is typically used to measure the sensitivity of an assay.

Singlet(s) A single microsphere or a population of single microspheres. Compare to doublets, which are two microspheres associated together.

Slope Defined as the rise over the run when plotting a series of points to form a line. Using the reporter validation kit, the slope of the response is related to the dynamic range of the array reader and yields information about the response of the photomultiplier tube (PMT).

Spectral address The unique fluorescent emission spectra of a microsphere.

Standard deviation The spread in individual data points (e.g., in a replicate group) that reflects the uncertainty of a single measurement.

Standard error The standard deviation of a set of replicates divided by the square root of the number of replicates.

Unconjugated microspheres A microsphere set that has been chemically modified so that its surfaces are covered with some functional group, but that has not been chemically prepared for use as a component in an assay system. These base microspheres do not carry biomolecular reactant.

Uniformity The reproducibility of a signal over a series of replicate measurements.

Validation A formal process for documenting that an instrument is fit for its intended use and is kept in an appropriate state of maintenance and calibration.

Validation kit A set of reagents and procedures designed to verify the performance of the Bio-Plex array reader apart from an assay. Validation kit 4.0 assesses the alignment of the optics assembly, measures the performance of the reporter channel, evaluates the fluidics system, and verifies the classify efficiency of the Bio-Plex array reader.

Variance The mean square deviation of a variable around the average value.

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Bulletin 2993 US/EG Rev B *04-0890 0105 Sig 1204*