TTP LABTECH

High-throughput antibody screening using high-sensitivity microplate cytometry

The increased focus on antibody-based therapeutics is driving a need for a faster, more robust alternative to ELISAs. Homogenous or 'mix-and-read' assays—popularized as FMAT® assays—overcome problems with automating ELISAs, particularly cell-based methods. TTP LabTech's Mirrorball® offers equivalent data with minimal change to existing assay protocols.

The study of antibodies has been a major focal point in biology and medicine for over 20 years. Since 2000 the therapeutic market for monoclonal antibodies (mABs) has grown exponentially, and they now have a major role in the treatment of a variety of disease states, including cancer and autoimmune diseases. mABs are routinely generated by hybridoma cell lines that secrete specific antibodies into the growth media; the rapid screening of these hybridoma supernatants for antibodies is central to efficient mAB generation.

Traditionally, the enzyme-linked immunosorbent assay (ELISA) was used for antibody screening. However, this tedious and time-consuming technique lacks the necessary sensitivity to be able to reliably and reproducibly detect low-abundance cell-surface proteins. Homogeneous mix-and-read assays overcome these problems. All of the assay components are added sequentially to one well, and analysis is performed once equilibrium has been reached. Because of their simplicity, mix-and-read assays are ideally suited to automation, although very few instruments have the sufficient sensitivity to analyze them. With increased interest in antibodies as therapeutics, there is an even greater demand for an instrument that has the necessary functionality and sensitivity to perform these assays.

Mirrorball® configuration

Mirrorball® is a unique laser-scanning fluorescence microplate cytometer that has been developed to facilitate antibody discovery. This system incorporates proprietary high-performance, low-loss optics that allow it to perform high-sensitivity mix-and-read assays for applications such as hybridoma screening. Unlike other microplate cytometers, Mirrorball offers simultaneous laser scanning, a capability that results in higher-throughput, single-pass scanning. This system also has an independent scatter channel, which permits label-free detection. This channel can run concurrently with fluorescence measurements, resulting in improved object recognition and fewer false negatives based on object count. Mirrorball is available in several configurations, with the highest specification offering dual laser excitation (488 nm and 640 nm), four fluorescence data channels and a single laser-scatter channel. The option of having dual laser excitation and four data channels extends the range of commercially available dyes that can be combined in a single assay, thus permitting superior multiplexing and making this a highly versatile system. Mirrorball can facilitate both cell- and bead-based assays. Cell-based assays can be conducted on live or fixed cells with adherent or suspension cell lines.

Label-free detection

Mirrorball is equipped with a single laser-scatter detector capable of recording signals from both 488 nm and 640 nm. The collection of laser scatter is independent of fluorescence detection and can be used to locate objects with little or no fluorescence. Mirrorball combines this label-free object recognition with simultaneous collection of up to four

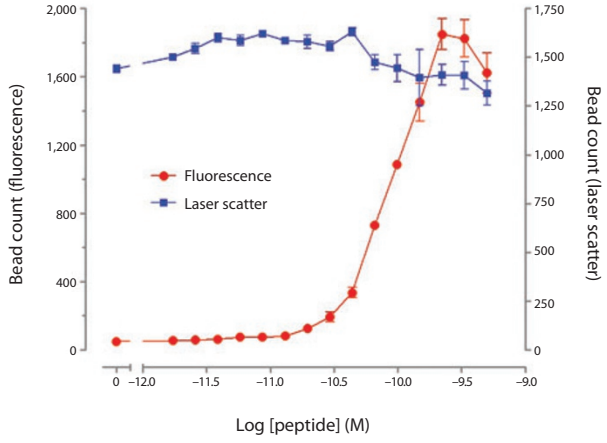

Figure 1 | Quantitative mix-and-read bead assay for a peptide. Laser scatter detects beads at all concentrations. In contrast, fluorescence-based recognition is limited.

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channels of fluorescence data for improved sensitivity in multiplexed assays. The utility of this functionality is illustrated in **Figure 1**. Using fluorescence, bead detection is very limited at levels below 20 pM, whereas the laser-scatter channel is able to detect all the beads in the sample, demonstrating how this channel can improve assay sensitivity significantly. Further improvements come from data normalization to bead count and reduction of false negatives from wells with reduced bead count.

Simultaneous laser scanning

Mirrorball is the first microplate cytometer to offer simultaneous scanning with multiple lasers. This is highly beneficial for mix-and-read assays, as it allows direct correlation of data across lasers, which permits superior multiplexing. This functionality increases throughput by eliminating the need for sequential scanning and improves assay performance because of the independent object recognition. The utility of this feature has been demonstrated in an antibody-binding assay for the epidermal growth factor receptor (EGFR). A549 cells were incubated with mouse anti-EGFR antibody and AlexaFluor 647®–labeled anti-mouse IgG antibody. They were also labeled with the lipophilic tracer DiO (for cell count). The simultaneous scanning capability allowed cell identification (DiO) and the amount of anti-EGFR labeling (AlexaFluor 647 fluorescence) to be determined concurrently. Whole-well images of the emitted fluorescence were created from the raw PMT readings (**Fig. 2**), and they demonstrate that the cell count was reasonably stable across the concentration range of the antibody. The anti-EGFR antibody-binding sensitivity was <5 ng/ml.

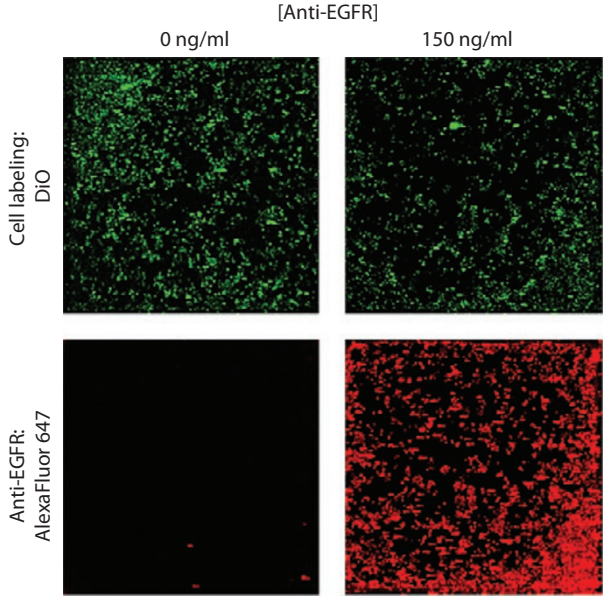

Figure 2 | Image set for dual laser scanning (488 and 640 nm). Whole-well images of the emitted fluorescence were created from the raw PMT readings and pseudo-colored. Top, cells stained with live cell stain DiO and scanned with a 488-nm laser. Bottom, corresponding data for anti-EGFR antibody simultaneously detected using 640-nm laser excitation.

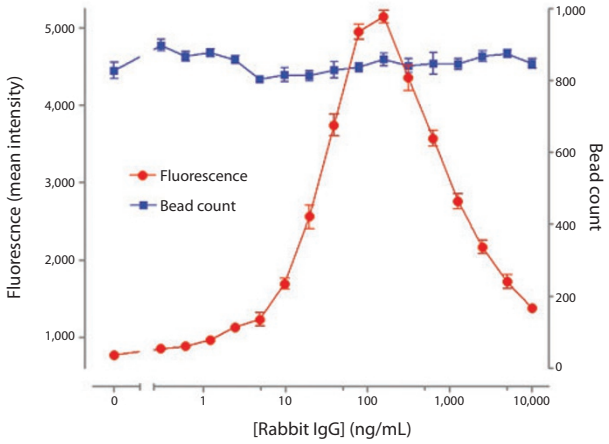

Figure 3 | Mix-and-read bead assay for rabbit IgG binding.

Bead-based assays

Homogeneous mix-and-read, bead-based assays are routinely used for antibody screening against soluble antigens. Such assays work on the premise that the beads are coated with specific antibodies, or antigens, of interest and then used as a binding surface in place of the microwells on the plate. Mirrorball offers advanced features as a mix-and-read analyzer for bead-based assays, and multiplexing can be achieved using beads of different sizes and fluorescence encoding. This system identifies beads through the application of cytometric analysis, which eliminates the need to wash away any unbound fluorescent agent and permits measurement of fluorescence bound to beads in the presence of the solution background. A bead-based immunoassay for rabbit IgG demonstrated that Mirrorball was capable of high-sensitivity detection of rabbit IgG, with a detection limit of <1 ng/ml (**Fig. 3**). A reduction in bead fluorescence was observed at concentrations above 150 ng/ml due to the 'hook effect'. The assay demonstrated that Mirrorball was capable of stable measurement of bead fluorescence despite the solution background.

Summary

Mirrorball is ideally suited to mix-and-read assays for the discovery of antibodies. Independent object recognition means that the user can be confident that false negatives are no longer a problem, and simultaneous scanning allows superior multiplexing for high-throughput, robust data generation. The versatility of this instrument provides cell-based assays that can be performed on live or dead cells, in adherent or suspension cultures. The ability to perform bead-based assays permits the detection of soluble antigens with high sensitivity.