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Advertorial: High-Throughput Cytokine Quantification Using Quantibody® Arrays

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For more than half a century, because of its superior specificity and sensitivity, the sandwich ELISA has been the gold standard for protein quantification. While ELISA has been a useful technique for detection of cytokines, unraveling the role of an individual cytokine in a physiologic or pathologic process often requires the consideration of a multitude of other molecules. In this regard, the limitations of ELISA are readily apparent and can quickly overwhelm the researcher who seeks to observe a large number of proteins. The labor and cost involved in processing a large number of plates, along with significant sample consumption are substantial hurdles for cytokine expression profiling.

To overcome these limitations, RayBiotech has developed a glass slide-based multiplex ELISA microarray platform: the Quantibody array (*Figure 1*). Each Quantibody slide is spotted with 16 identical cytokine antibody arrays. Each array contains two positive controls and up to 40 different cytokine-specific capture antibodies, all arrayed in quadruplicate. Target cytokines trapped on the solid surface are then detected using a laser scanner after the addition of a cocktail of biotin-labeled detection antibodies and a streptavidin-labeled Cy3 equivalent flour. Serial dilutions of a predetermined calibration standard mix are then used to generate a standard curve for each cytokine.

The Quantibody slide comes with a 16-well removable gasket and chamber assembly that accommodates 8 standards and 8 samples (or up to 16 samples) per slide. To increase throughput, 4 slides can be nested into a tray matching the dimensions of a standard ELISA microplate, thus allowing automated robotic processing of 64 arrays simultaneously.

Sensitivity and Specificity

Quantibody arrays are manufactured with a noncontact piezoelectric arrayer. During the array development process, all the antibodies and standards within the same array are rigorously tested to ensure negligible cross reactivity. The detection sensitivities of specific cytokines in Quantibody arrays were evaluated by comparison with ELISA (*Figure 2*). To ensure data quality, the same pairs of antibodies together with the same antigen sources were employed on the Quantibody slides and ELISA plates. Standard protocols were followed using the same reagents, personnel, and with equipment appropriate to each platform. Results show that the Quantibody

fluorescence detection has a larger dynamic range (by 2-logs) compared with the traditional colorimetric ELISA (*Figure 2A*); there is also a strong correlation in detection levels between Quantibody and ELISA (*Figure 2B*).

Stability and Reproducibility

In order to evaluate stability and reproducibility of the Quantibody platform, six Quantibody Human TH1/TH2 array kits manufactured at different times (ranging from 1 to 20 months prior to the experiment) were used simultaneously to determine cytokine concentrations using aliquots of the same samples. After parallel processing of the experiments, fluorescent signal intensities were obtained by scanning the slides using identical equipment and parameters. The extracted fluorescence signals of the 8-point standards from all six slides were highly comparable with an average correlation of 98.5%. Subsequently, the cytokine concentrations were calculated and found to exhibit an inter-assay CV of approximately 12% on average for 10 cytokines on six different slides.

Accuracy and Precision

The system CV was assessed using the Quantibody Human TH1/TH2 array kit. In this study, four wells of the same sample were run in parallel along with the standards by two distinct researchers on two different occasions. The average intra-assay CV was found to be 6.6% by researcher 1 and 10.9% by researcher 2, while the average inter-assay CV was 12.4%. Individual Reference Standard Antigens were analyzed to determine system accuracy in repeat testing. The Human TH1/TH2 array correctly detected the projected cytokine concentration with a standard deviation of ~8%.

Recovery

Recovery is the extraction efficiency of an analytical method within the limits of experimental variability. The applicability of Quantibody for analyzing serum and cell culture medium was evaluated by spiking different levels of recombinant proteins into the matrix. Using a nonspiked sample as negative control, the recovery rate for each cytokine was then determined by subtracting the endogenous cytokine level from the observed value and divided by the spiking cytokine concentration. The average media recovery of Quantibody human TH1/TH2 array was 105±14% for serum and 96±14% for culture media.

Quantibody Products and Applications

RayBiotech has over 800 validated antibody pairs in the Quantibody line, detecting a broad range of targets including cytokines, chemokines, growth factors, receptors, proteases, and other regulatory factors. Quantibody arrays are fully customizable, and the line includes an assortment of premade arrays for diverse research topics such as inflammation, angiogenesis, neuroscience, cardiovascular disease, cancer, sepsis, obesity, dry eye disease, and periodontal disease. Currently RayBiotech carries Quantibody kits that allow the detection of 400 human, 200 mouse, 100 rat, 80 monkey, 20 porcine, 20 canine, 20 bovine, 10 equine, or 10 feline cytokines in a single experiment.

Automated processing of Quantibody arrays can be achieved with most liquid-handling systems, enabling not only increased throughput, but also significant improvement of both intra- and inter-assay repeatability (CV <10%). At the same time, such automation allows one technician to quantitatively detect hundreds of proteins in hundreds of samples in a single day, making the Quantibody platform ideal for use in clinical trials or biomarker discovery.

With references in hundreds of peer-reviewed research articles, Quantibody represents a powerful tool for identification of crucial factors involved in disease processes, validation of gene microarray results, identifying potential molecular targets for drug development, and identifying the molecular mechanisms of drug action.

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