Canon PR610-2 Fluorescence Immunoassay Optical Waveguide System

Waveguide Antibody Coating Procedure:

Clean the waveguides by removing the waveguides from their holder and washing them in isopropanol with the following procedure, or an equivalent.

Place them at every other position in an empty Eppendorf pipette tip box. Fill the waveguide-containing box with isopropanol up to the holes in the tip holder and sonicate the waveguides in their holder using a Branson 5510 sonication bath for 30 seconds. Discard the isopropanol and rinse the waveguides 3 times with DI water. Air-dry the washed waveguides and use them within 3 days.

The dried waveguides can be antibody coated by soaking in 5 microgram / ml affinity purified polyclonal or monoclonal antibody in 0.1 M pH 9.6 Na HCO_3 buffer containing 5 % Glycerol (W/V) for 1 hr at room temperature or overnight at 4C. To minimize antibody consumption, small volume tubes can be made by flame or wax sealing the ends of epT.I.P.S. 0.5-20 uL Eppendorf pipette tips. These tubes hold 0.1 ml and can be filled from the bottom up with a microcapillary 1-200-uL-Gel tip. Store the coated waveguides at 4C above a desiccant.

Fluorescent Signal Antibody Preparation:

The fluorescent signal antibody can be prepared using CY5 (Amersham Biosciences PA25001) or Alexa Fluor 647 labeling kits (Molecular Probes A-20186). Follow the provided instructions. The labeled antibody can be used in an assay at 5-10 micrograms per ml in PBS 0.05% Triton X100 buffer.

Assay Protocol Examples:

The Canon PR6102 system allows three protocols labeled FL, BG, and SG. Each protocol can be selected to repeat up to 3 times and all three protocols can be selected at the same time if desired. Protocol FL is used to determine the background fluorescence of between 1-4 optical waveguides. The protocol will pick up a coated waveguide from the middle Well position and move it to the Read/Wash position and the instrument then washes the fiber for the time set in the “Rinse time” window (usually 15 seconds) and then the fluorescence is recorded for 20 seconds. The instrument then replaces the fiber into its original position. It will then pick up the next waveguide and repeat the process for as many Sample numbers as selected (1-4). A wash buffer containing 0.05% Triton X100 in PBS is satisfactory. This wash buffer should also be placed in the middle Well position to rehydrate the dried-coated waveguide.

The BG protocol is used to determine any non-specific binding of the fluorescent antibody to the coated waveguide. In this protocol a waveguide is picked up from the middle Well and moved to the back Well which contains the fluorescent labeled antibody. It incubates with the antibody for the time designated in the “Labeled antibody incubation time” window. If four samples are being assayed this time needs to be at least five minutes (300 seconds). Next the waveguide is picked up and moved to the Read/Wash position and washed for the indicated time and then the fluorescence is recorded. The waveguide is then moved back to its original position. The next waveguide is then processed for as many sample numbers

as selected (1-4). The incubation times are interleaved such that the total assay time is much less than 20 minutes for 4 assays at 5 minutes each.

The SG protocol is used to perform an immunoassay. In this protocol the waveguide is picked up from the middle position and moved to the front position where it incubates with the sample antigen. The time of this incubation is programmed in the "Specimen incubation time" window. Fifteen minutes is usually satisfactory for high affinity antibodies and 10-1000 ng/ml antigen. After this incubation the waveguide is moved to the Read/Wash position and washed for the indicated time and the background fluorescence recorded. The waveguide is then moved to the back Well containing the fluorescent antibody and incubated for the programmed time (usually 5-min). The waveguide is then moved back to the Read/Wash position, washed, and then the antigen bound fluorescence is recorded. The waveguide is then returned to its starting position. The next waveguide is then processed and again the incubation times are interleaved such that the total assay time is much less than 60 minutes for 4 assays at 15 minutes each. The background fluorescence is subtracted from the sample fluorescence. A calibration curve with known amounts of antigen needs to be determined with each assay batch.

Goat IgG Assay Example:

Canon Optical waveguides were coated with 5 micrograms per ml AffiniPure Rabbit anti-Goat IgG (305-005-003, Jackson Immuno Research Laboratories Inc.) as described above. Ten ml of ChromoPure Goat IgG [0.74-20 ng/ml in Assay Wash (PBS/0.05% Triton X100)] were placed in the first of four Wells. Also 0.4 ml of Assay Wash were placed in the second Well along with an anti-Goat IgG coated waveguide. Finally 0.2 ml of 5 micrograms per ml of CY5 labeled Rabbit anti-Goat IgG (305-175-003, Jackson Immuno Research Laboratories Inc.) in Assay Wash were placed in the third Well. The assay was started using the SG protocol with a five-minute incubation time for both the antigen and the labeled antibody binding steps. A 15-second Rinse Time using Assay Wash was used prior to each fluorescent read. Figure 1 illustrates the linearity of the results.