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EXAMPLE #1

PREPARATION OF ANTIBODY COATED GLASS COVERS LIP

A glass coverslip previously boiled in 10% nitric acid and washed in distilled water was coupled to mouse monoclonal anti-BSA or non-specific antibody through a silane coupling agent previously attached to the glass surface. The coverslip containing immobilized antibody was washed to remove unbound antibody and could be stored under suitable conditions until further use.

To decrease the non-specific attachment of antigen-coated beads to the glass coverslip, the coverslips are preferably prepared using a mixed silane solution comprising 85–99% by volume of an alkylsilane and 1–15% solution of a silane functionalized for coupling of protein. The functionalized silane has a functional group capable of forming a covalent bond with protein, e.g., epoxy, amine, carboxyl, or hydroxy, and may be selected from the group of heterobifunctional cross-linking agents and homobifunctional cross-linking agents. Such agents are well known in the art. This preferred method of preparing the coverslips is described in more detail in Example 1A,

EXAMPLE #1A

PREPARATION OF COVERS LIPS FOR COVALENT COUPLING OF ANTIBODY

Glass coverslips were cleaned by boiling in 10% nitric acid for 1 hour and washed in distilled water until the wash had a neutral pH. Silane solution was prepared by adding 5 ml of 4-glycidioxypropyltrimethoxysilane (GPTMS) and 0.05 to 5 ml of tetramethyl orthosilicate (TMOS) to 100 ml deionized water. The pH of the silane solution was adjusted to pH 4.0 with hydrochloric acid solution. Glass coverslips were dipped into the silane solution and dried at room temperature. They were then heated in a vacuum oven to 110° C. for 90 minutes. The resulting coated coverslips were then used for covalent attachment of the antibody. The decrease in non-specific binding of antigen to the glass coverslips is seen by comparing the data in FIG. 4 with FIG. 2.

EXAMPLE #2

PREPARATION BSA COATED LATEX BEADS

Polystyrene beads (varied between about 0.5 μ to about 100 μ in diameter), carrying carboxyl groups were coupled to BSA using water soluble carbodiimide. The quantity coupled was decreased in 10-fold steps from 1.49 $\times 10^{-5}$ M to a final concentration of 1.49 $\times 10^{-15}$ M. These beads were washed to remove uncoupled BSA and stored in 0.05M sodium phosphate buffer, pH 6.6, until used.

EXAMPLE #3

DETECTION OF SINGLE ANTIGEN/ANTIBODY BOND

Antigen-coated latex beads (from Example 2) were placed on the antibody-coated coverslip (from Example 1 or 1A) and the force required to optically trap the beads was determined. The antigen/antibody pair was either BSA/anti-BSA to represent a specific binding pair or BSA/nonspecific immunoglobulin as a nonspecific control. At each antigen concentration a minimum of ten beads were tested. The results are shown in FIGS. 2–4. As can be seen in FIG. 4, the average value for the force required to move the BSA-coated bead in the BSA/anti-BSA assay was greater than that required in the nonspecific control assay. Thus, the specific

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difference between the BSA/anti-BSA and control power values at a given BSA concentration represents the power required to break specific antigen-antibody bonds at that BSA concentration, with the specific difference at the concentration of BSA representing one BSA molecule per bead (compare FIG. 3 and 4) representing the force required to break a single, specific antigen/antibody bond. The ability to detect specific antigen-antibody binding was also tested by comparing BSA/anti-BSA with other nonspecific controls, i.e., BSA/silane, uncoated beads/anti-BSA, and BSA/anti-BSA pretreated with a vast excess of BSA. These assays gave similar results (data not shown).

EXAMPLE #4

DETECTION OF SUB-ZEPTOMOLES OF ANTIGEN

The BSA/Anti-BSA system described in Example 3 was operated in the presence of decreasing concentrations of BSA in solution using 1 microliter samples added to a 100 μ l of buffer in a well. Using a concentration of 1 molecule of BSA/1 microliter of sample, the system could detect 1.6 $\times 10^{-18}$ is Molar BSA by competitive binding. This approach will be applicable to any antigen-antibody system regardless of the nature of the antigen. Using BSA coated particles having different BSA concentrations yielded different sensitivities as shown by the displacement bar graphs in FIG. 5.

EXAMPLE #4A

DETECTION OF ANTIGEN IN SERUM

Human serum was chosen as the diluent for this set of experiments. The antibodies used for the study were the same monoclonal antibodies used in the other examples. Known quantities of BSA were diluted into serum as follows: BSA in a volume of 1 μ l was added to 100 μ l of human serum to a final selected concentration. The assay was carried out as described in previous examples except that the serum sample was substituted for BSA diluted in buffer as used in Example #4. The results of these experiments are presented in the table. The standard curve was derived from replotting the bar graphs (FIG. 5). The BSA-coated beads containing 1.49 $\times 10^{-7}$ moles/L of BSA were used unless otherwise noted.

Sample #	Theoretical Value (moles/L)	Calculated value from Std. Curve
1	1.49 $\times 10^{-10}$	1.25 $\times 10^{-10}$
2	1.49 $\times 10^{-10}$	1.62 $\times 10^{-10}$
3	1.49 $\times 10^{-10}$	1.52 $\times 10^{-10}$
4	1.49 $\times 10^{-13}$	1.65 $\times 10^{-13}$
5	1.49 $\times 10^{-13}$	1.80 $\times 10^{-13}$
6	1.49 $\times 10^{-13}$	2.00 $\times 10^{-13}$
7	1.49 $\times 10^{-15}$	1.58 $\times 10^{-15}$ *
8	1.49 $\times 10^{-15}$	1.42 $\times 10^{-15}$ *
9	1.49 $\times 10^{-15}$	1.45 $\times 10^{-15}$ *

* Assay performed with beads coated at level of 1.49 $\times 10^{-13}$ moles/L of BSA.

This data indicates that it is possible to assay a serum sample containing specific antigen and detect and quantitate the analyte therein. It is also obvious to anyone expert in the state-of-art, that one can substitute antibody for antigen in a similar assay with similar results. In this case, the added antibody would compete with the immobilized antibody for the available antigen attached to the beads.