

alkaline phosphatase enzyme as the conjugate 24, and 5-bromo-4-chloro-3-indolylphosphate (BCIP) as the substrate, S. The PV-Fc layer serves as a hydrophobic layer, enhancing adsorption of the anti-APS reductase antibody.

The first step was adsorption of the anti-APS reductase antibody onto a PV-Fc layer, applied by spin coating from a 10% o-chlorotoluene solution onto the gold/quartz surface 18 of a quartz crystal. This was performed by equilibration of the PV-Fc modified gold/quartz crystal with 1.5 mL of 100 ug/mL anti-APS reductase antibody in PBS buffer solution for 2 hours. The crystal was then washed 3 times with PBS buffer to remove excess anti-APS reductase antibody. The crystal was then exposed to 0 to 80 mL of APS reductase (0.5 mg/mL) in 2 mL TRIS test buffer solution for 30 minutes; the dosage rate was varied to determine the response characteristics of the device. After being washed with TRIS test buffer to remove nonspecifically adsorbed APS reductase, the crystal was exposed for thirty minutes to 1.5 mL TRIS test buffer solution containing 30 mL of conjugate comprised of anti-APS reductase antibody and alkaline phosphatase enzyme. The crystal was then washed again with TRIS test and TRIS wash buffers as described in Example 1.

The detection step was performed by immersing the crystal in 0.5 mL of TRIS buffer in a cell holder, followed by addition of 0.5 mL of a standard detection solution containing 50% dilution of BCIP substrate reagent (SIGMA) in 50 MM TRIS buffer (SIGMA). A positive response for antigen was measured by the decrease in frequency, corresponding to the precipitation of the oxidized dimer of BCIP, an indigo dye analog. Precipitation results from the enzymatically catalyzed hydrolysis of the phosphate functionality of BCIP, which, in turn, only occurs if the alkaline phosphatase conjugate is present, which, in turn, is only possible when the APS reductase is present. Table 2 indicates the frequency response of the BMQCM to different dosage levels of APS reductase. It is clear that the frequency change and the rate of change increase with larger dosage rates, as expected. The relative responses agreed with those determined by the spectroscopically measured optical density of the blue BCIP indigo dimer deposited on the surface 18 of the quartz crystal microbalance.

TABLE 2

APS reductase concentration (ng/mL)	Δ frequency/sec (Hz/sec)	Δ frequency in 30 min.
200	0.22	278
75	0.09	80
25	0.05	69
7.5	0.025	22

EXAMPLE 3

Assay of human chorionic gonadotropin (hCG) using horseradish peroxidase/hydrogen peroxide-iodide on nylon membranes situated opposite to a quartz crystal microbalance modified with polyvinylferrocene modifying layers

The procedure was performed according to the mode illustrated in FIGS. 4 and 6, with a polyvinylferrocene (PV-Fc) layer 28 on the quartz crystal situated opposite to a nylon membrane 36 whose surface 30 was modified with capture reagent. The spacing of the compartment was approximately 1 mm. In this example, anti-hCG

antibody was the capture reagent 20, hCG was the analyte 22, anti-hCG antibody with horseradish peroxidase (HRP) enzyme was the conjugate 24, and a hydrogen peroxide-iodide mixture was the substrate, S. The nylon membrane served as a hydrophobic layer that adsorbed the anti-hCG antibody, and presented the enzymatic reaction product to the PV-Fc film. The enzymatically catalyzed formation of iodine/triiodide that occurred when the conjugate was present resulted in oxidation of the PV-Fc film, followed by incorporation of triiodide into the film to maintain electroneutrality. This led to an increase in mass of the PV-Fc film and accordingly a large change in the resonant frequency of the BMQCM device.

The first step was adsorption of the anti-hCG antibody to the nylon surface 30 of membrane 36. This was performed by equilibration of the nylon membrane 30 with 2 mL of 100 ug/mL anti-hCG antibody in PBS buffer solution for 2 hours. The membrane was then washed once with PBS buffer containing 0.1% BSA and then 3 times with PBS buffer to remove excess anti-hCG antibody and block any nonspecific binding sites. The membrane was then exposed for thirty minutes to different concentrations of hCG in PBS buffer containing 0.1% BSA buffer solution. After being washed with PBS buffer to remove nonspecifically adsorbed hCG antigen, the membrane was exposed for twenty minutes to 2 mL buffer solution containing 20 mL of stock conjugate comprised of anti-hCG antibody and HRP enzyme. The membrane was then washed again with PBS/0.1% BSA buffer solution.

The detection step was performed by placing the membrane opposite to the quartz crystal modified with the PV-Fc film, with a 1 mm diameter separator between the two surfaces to form a reaction compartment. The compartment was filled with a pH=5.0 citrate/-phosphate/iodide detection buffer. After the frequency stabilized, 10 mL of a standard solution of 0.01% hydrogen peroxide was added. The enzymatically catalyzed reaction product iodine/triiodide, P, diffuses across the compartment to the PV-Fc film, resulting in oxidation of the PV-Fc film by one-half of an equivalent of P. This is subsequently followed by incorporation of an equivalent of triiodide into the oxidized PV-Fc film, resulting in an increase in mass of the film and a corresponding decrease in resonant frequency of the quartz crystal microbalance. Triiodide incorporation results from the enzymatically catalyzed conversion of iodide to iodine/triiodide, which, in turn, only occurs if the HRP conjugate is present, which, in turn, is possible only when the hCG antigen is present.

TABLE 3

hCG concentration (ng/ml)	Δ frequency/sec (Hz/sec)	Δ frequency in 10 min.
0	<0.005	<3
600	0.01	48

The invention is defined by the following claims, although it will be appreciated by those skilled in the art that various modifications can be made without departing from the spirit thereof.

What is claimed is:

1. In a process for detecting an analyte suspected of being present in a liquid sample, which process comprises reacting the sample with a quartz crystal microbalance (QCM) having an analyte capture reagent