

buffer to the BMQCM/analyte assembly at room temperature. Conjugate can be prepared, for example, according to the method of Imagawa [J. Biochem., 92, 1413 (1982)] or Avrameas et al. [Scand. J. Immunol., Vol. 8, Suppl. 7, 7-23 (1978)] and maintained at 4° C. as a stock solution. Conjugates of enzymes and synthetic polynucleic acids can be prepared according to the method of Ruth et al. [DNA, 4, 93 (1985)]. This is then followed by washing with TRIS test buffer solution to remove nonspecifically adsorbed conjugate 24. The total amount of conjugate 24 should be added at a concentration to exceed the amount of specifically adsorbed analyte 22. Alternatively, the analyte and conjugate may be added simultaneously.

In a so-called competitive mode, the analyte and an analyte/enzyme conjugate may be reacted with the BMQCM. The analyte and conjugate may be added sequentially or simultaneously.

The frequency of the quartz crystal microbalance is measured in 50 MM TRIS wash buffer solution, and then a standard solution of the substrate is directly added. The rate of frequency change, as well as the total frequency change after a time considered to be the optimum measurement interval, are measured in solution and, since conjugate is present only when analyte is bound to the surface, are indicative of the amount of analyte exposed to the BMQCM. The signal measured is amplified by the large turnover numbers of the enzymatic reaction, which produces concentrations of product far exceeding that of the analyte.

The present invention can be embodied in diagnostic kits comprising crystals treated with the desired capture reagent and any modifying layers 28, and an oscillator circuit with direct readout of the resonant frequency of the quartz crystal microbalance. In typical use, the analyte solution, for example patient serum, would be added to a compartment containing the BMQCM, followed by a wash with buffer solution, followed by addition of the appropriate enzyme/anti-analyte reagent conjugate, followed by a wash. The substrate would then be added and the frequency change directly measured. The preferred mode of operation would include the use of a reference crystal which is exposed to the identical solutions, but which has not been modified so as to have capture reagent on or in proximity to its surface. In this manner, the difference in frequency between the sample and reference crystals can be measured and errors due to changes in viscosity, temperature and non-specific binding minimized. Operation with only the sample crystal, however, is also feasible, because (1) the viscosity and temperature changes during addition of the substrate and during measurement are not large, (2) interference from nonspecific adsorption is minimized by the washing step, and (3) the measurement step poses no risk of mass changes from processes other than those induced by P.

The invention is further illustrated by the following nonlimiting examples.

EXAMPLE 1

Assay of adenosine-5'-phosphosulfate (APS) reductase using alkaline phosphatase/5-bromo-4-chloro-3-indolylphosphate (BCIP) on unmodified quartz crystals

The procedure was performed according to the mode illustrated in FIG. 1, with anti-APS reductase antibody as the capture reagent 20, APS reductase as the analyte 22, anti-APS reductase antibody with alkaline

phosphatase enzyme as the conjugate 24, and 5-bromo-4-chloro-3-indolylphosphate (BCIP) as the substrate, S. The first step was adsorption of the anti-APS antibody on the gold/quartz surfaces of a quartz crystal. This was performed by equilibration of the gold/quartz crystal with 2 mL of 100 ug/mL anti-APS reductase antibody in PBS buffer solution for 2 hours. The crystal was then washed once with PBS buffer containing 0.1% bovine serum albumin (BSA) and then 3 times with PBS buffer to remove excess anti-APS reductase antibody and block any nonspecific binding sites. The crystal was then exposed to varied concentrations of APS reductase ranging from 0 to 400 ng in 1.5 mL of TRIS test buffer solution for 20 minutes; the dosage concentrations were 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 twenty minutes to 1.5 mL TRIS test buffer solution containing 30 mL conjugate comprised of anti-APS reductase antibody and alkaline phosphatase enzyme. The crystal was then washed again 2 times with test buffer and once with 50 MM TRIS wash solution.

The detection step was performed by immersing the crystal in 0.5 mL of TRIS wash buffer in a cell holder, followed by addition of 0.5 mL of a standard solution of BCIP reagent solution (SIGMA). A positive response for antigen was measured by a 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 1 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. Notably, these responses were observed for APS reductase levels in which the direct binding of APS reductase could not be observed. That is, the frequency change resulting from addition of APS reductase to the BMQCM was not detectable.

TABLE 1

APS reductase concentration (ng/mL)	Δ frequency/sec (Hz/sec)	Δ frequency in 30 min.
200	0.24	406
75	0.17	250
25	0.10	170
7.5	0.002	6.3

EXAMPLE 2

Assay of APS using alkaline phosphatase/5-bromo-4-chloro-3-indolylphosphate (BCIP) on quartz crystals with polyvinylferrocene modifying layers

The procedure was performed according to the mode illustrated in FIG. 2, with a polyvinylferrocene (PV-Fc) layer 28 on the quartz crystal, an anti-APS reductase antibody as the capture reagent 20, APS reductase as the analyte 22, anti-APS reductase antibody with