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Patents and Literature

Bioassays

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ABSTRACT

Bioassays, including immunoassays, enzyme assays, and assays using enzyme electrodes, and nucleic acid hybridization probes have been the subject of considerable industrial and academic research. New bioassay methods have applications in the medical, chemical, pharmaceutical, and food products industries. Recent US patents and scientific literature on a variety of new bioassay methods are surveyed. A description of these patents and a list of references are given.

INTRODUCTION

The objective of the Patents and Literature Section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnological opportunities. Three subject areas have been surveyed in 1987: immobilized biocatalysts; monoclonal and immobilized antibodies; and bioassays based on immunological, enzyme, gene probe, and electrochemical methods. The subject of this, the last Patent and Literature Section of 1987, is Bioassays.

PATENTS

This section identifies and gives a brief description of patents from US patent literature from September 1985 to May 1987. The major search heading was assay with the crossterms: immuno, enzym, and bio. In addition, enzym and electrod and hybridiz and probe(s) were also searched. Both patent abstracts and titles were searched. Copies of US Patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC, 20231.

HYBRIDIZATION PROBES

Albarella, J. P., and Anderson, L. H. D.
NUCLEIC ACID HYBRIDIZATION ASSAY EMPLOYING ANTIBODIES TO INTERCALATION COMPLEXES
US 4563417, Jan. 7, 1986
Assignee: Miles Laboratories, Inc.

A nucleic acid hybridization assay for detecting a particular polynucleotide sequence in a test medium is described. An aggregate is formed in the assay reaction mixture between a nucleic acid intercalator and double stranded nucleic acid associated with the hybridization product of the sequence to be detected and a nucleic acid probe sequence. Hybridization of the probe with the sequence to be detected can then be determined by addition of an antibody capable of binding with the intercalation complexes in the formed aggregate. This method eliminates the need to chemically modify the probe in order to form a labeled reagent.

Paau, A., Platt, S. G., and Sequeira, L.
ASSAY METHOD AND PROBE FOR POLYNUCLEOTIDE SEQUENCES
US 4556643, Dec. 3, 1985
Assignee: Agracetus

An assay method for the detection of a specific nucleotide target sequence in a polynucleotide extract is described that utilizes a polynucleotide modified probe including both a cDNA sequence substantially complementary to the specific target sequence and a protein binding sequence. The modified probe is exposed to the polynucleotide test extract for hybridization and then the complex is exposed to the protein that binds to the protein binding sequence. An immunoassay can then be conducted to indicate the presence of the specific target by detecting the presence of the binding protein.

Ranki, T. M., and Soderlund, H. E.
DETECTION OF MICROBIAL NUCLEIC ACIDS BY A ONE-STEP SANDWICH HYBRIDIZATION TEST
US 4563419, Jan. 7, 1986
Assignee: Orion Corporation Ltd.

A kit is described for the detection and identification of microbial nucleic acids using a one-step sandwich hybridization technique. The technique requires two complementary nucleic acid reagents for each microbe or group of microbes to be identified.

Sheldon, E. L., III., Levenson, C. H., Mullis, K. B., and Rapoport, H.
PROCESS FOR LABELING NUCLEIC ACIDS USING PSORALEN DERIVATIVES
US 4582789, Apr. 15, 1986

and

Sheldon, E. L., III., Levenson, C. H., Mullis, K. B., Rapoport, H., and Watson, R. M.
PROCESS FOR LABELING NUCLEIC ACIDS AND HYBRIDIZATION PROBES
US 4617261, Oct. 14, 1986
Assignee: Cetus Corporation

A labeling reagent is prepared which contains an alkylating intercalation moiety, such as 4,5′, 8-trimethylpsoralen, a divalent organic spacer arm moiety and a monovalent label moiety capable of producing a detectable signal. This reagent may be used to label DNA, by intercalating into double-stranded nucleic acid to form a complex and activating the complex forming a covalent bonding between the reagent and the nucleic acid. The labeled nucleic acid is a hybridization probe capable of detecting a specific nucleic acid sequence, and hybridizing with it. This reagent may also be used in chromosome banding to lable specific regions of chromosomes and thereby differentiate them.

Yabusaki, K. K., Isaacs, S. T., Gamper, Howard, B., Jr.
NUCLEIC ACID HYBRIDIZATION ASSAY EMPLOYING PROBES CROSSLINKABLE TO TARGET SEQUENCES
US 4599303, July 8, 1986
Assignee: HRI Associates, Inc.

The presence of specific nucleic acid base sequences are determined by employing crosslinking reactions of unique molecules capable of forming covalent bonds that are bonded with various labels or ligands for amplification. Single stranded nucleic acid probes are employed that contain complementary base sequences to nucleic acid target molecules. By first hybridizing and then forming covalent bonds between the probe and the target, the amount of label in the crosslinked hybrid can be measured. This represents an extremely sensitive method for assaying for specific nucleic acid sequences.

IMMUNOASSAYS

Armenta, R. D., and Gibbons, I.
IMMUNOASSAYS EMPLOYING PROTECTED LABELS
US 4578350, Mar. 25, 1986
Assignee: Syntex (USA), Inc.

An improved immunoassay is described for determining the presence of an analyte in a serum sample. The sample is combined with a conjugate of the analyte and an enzyme and with a receptor for analyte, and the presence of analyte in the sample is determined from the effect that the

sample has on the enzymatic activity when compared to the enzymatic activity in the absence of analyte or in the presence of known amounts of analyte.

Baker, T. S., Abbott, S. R., Simpson, J. G., Wright, J. F., and Powell, M. J.
HETEROGENEOUS BINDING ASSAY
US 4656143, Apr. 7, 1987

A heterogeneous binding assay is described in which a liquid component and a granular particulate solid phase are incubated together for a predetermined period of time. To prevent unwanted gravity sedimentation of the solid phase during incubation, the density of the liquid component is controlled such that it is equal to the density of the granular particulate solid phase. The density is controlled by adding a density modifying medium such as a colloidal suspension of silica particles coated with polyvinylpyrrolidine.

Baldeschwieler, J. D., Gamble, R. C., Lin, A. M., and Tin, G. W.
MEMBRANE IMMUNE ASSAY
US 4581222, Apr. 8, 1986
Assignee: California Institute of Technology

An immunoassay method for detection of antigen is described. The method employs complement mediated lysis of vesicles loaded with In-111 or other gamma-emitting cation, and quantitative detection of the lysis by gamma-ray perturbed angular correlation (PAC) spectroscopy.

Baldwin, T. O., Holzman, T. F., Satoh, P. S., and Yein, F. S.
IMMUNOASSAYS WITH LUCIFERASE LABELED LIGANDS OR RECEPTORS
US 4614712, Sep. 30, 1986
Assignee: The Upjohn Company: Texas A&M University System

Immunoassays are described which utilize an enzyme linked ligand or receptor where the enzyme is bacterial luciferase.

Block, M. J., and Hirschfeld, T. B.
APPARATUS INCLUDING OPTICAL FIBER FOR FLUORESCENCE IMMUNOASSAY
US 4582809, Apr. 15, 1986
Assignee: Myron J.

A method and apparatus for fluorescent immunoassay is described that utilizes total internal reflection at the interface between a solid phase and a liquid phase of lower index of refraction to produce an evanescent wave in the liquid phase. The solid phase is arranged and illuminated so as to provide multiple total internal reflections at the interface. The solid phase

is an optical fiber to which is immobilized a component of the complex formed in the immunochemical reaction. A fluorophore is attached to another component of the complex. The fluorescent labeled component may be either the complement to or an analog of the immobilized component, depending on whether competitive or sandwich assays are to be performed. The fiber (and its attached constituent) is immersed in the liquid phase sample. The evanescent wave is used to excite fluorescence in the liquid phase, which tunnels back into the solid phase and accumulates during multiple reflections. Diffusional preconcentration onto the reactive surface and double passing enhance the signal and signal/background ratio.

Burton, J. A., and Hoop, B., Jr.
METHOD AND APPARATUS FOR LIGAND DETECTION
US 4626513, Dec. 2, 1986
Assignee: Massachusetts General Hospital

A process and apparatus has been developed for radioassay of ligand in solution, eliminating the separation step required in conventional techniques. A chamber contains a quenching solution, ligand molecules, and receptor molecules. One forms a free species labeled with a beta particle emitter, whereas the other is immobilized on a solid support. Ligand introduced with the sample competes with ligand molecules already in the chamber for receptor sites on the receptor molecules and the free species is allowed to diffuse about the chamber. A beta particle detector in communication with the chamber at a fixed position detects only those beta particles emitted from within the quenching distance of the quenching solution. The quenching properties of the solution are used in place of the conventional separation step.

Chagnon, M. S., Groman, E. V., Josephson, L., and Whitehead, R. A.
BINDING ASSAYS EMPLOYING MAGNETIC PARTICLES
US 4628037, Dec. 9, 1986
Assignee: Advanced Magnetics, Inc.

A process is described for the preparation of magnetic particles to which a wide variety of molecules may be coupled. The magnetic particles can be dispersed in aqueous media without rapid settling and conveniently reclaimed with a magnetic field. The particles do not become magnetic after application of a magnetic field, can be reused, and are useful in biological systems involving separations.

Chandler, H. M., Healey, K., and Hurrell, J. G. R.
METHOD FOR DETECTING ANTIGENS AND ANTIBODIES
US 4590157, May 20, 1986
Assignee: Commonwealth Serum Laboratories Commission

A method is described for detecting the presence of antigens or antibodies in a sample by an enzyme-linked immunosorbent assay using urease, urea, and di-bromo-cresolsulfonphthalein as the indicator.

Chang, T. W.
MATRIX OF ANTIBODY-COATED SPOTS FOR DETERMINATION OF ANTIGENS
US 4591570, May 27, 1986
Assignee: Centocor, Inc.

A novel immunoassay device is described for the determination of antigenic substances that comprise a pattern or array of minute antibody-coated spots on the surface of a support. The array of antibody-coated spots, preferably in the form of a rectangular matrix, is made up of antibodies of a different and distinct specificity corresponding to each spot. A large number of different antibody-coated spots can be assembled on a very small portion of the surface of the support. The spots serve as tiny, specific immunoabsorbents of cells. The expression of particular surface antigen by cells may be detected by determining to which antibody-coated spot the cells bind.

Deutsch, A., and Dorsey, N.
IMMOBILIZED PROTEIN ON NYLON FOR IMMUNOASSAY
US 4615985, Oct. 7, 1986
Assignee: Genetic Diagnostics Corp.

A partially hydrolyzed nylon matrix having free amino groups is coupled to a protein molecule by a divalent radical that is the residue of a diimide molecule. The nylon carrying the protein can then be used in an immunoassay or an enzyme assay.

Diamond, S. E., and Regina, F. J.
BINDING ASSAY WITH AMPLIFIED READ-OUT AND GAS-PHASE DETECTION
US 4629689, Dec. 16, 1986
Assignee: Allied Corp.

At the inclusion of a selective binding assay an enzyme is present in modulated concentration and/or activity to moderate a chemical reaction such as the cleavage of o-nitrophenyl-beta-D-galactopyranoside. After a controlled period, the enzymatic product is transferred to the gas phase and concentrated relative to other components in the enzymatic reaction mixture and injected into a gas chromatography column and detected by flame ionization or electron capture.

Frey, W. A., and Simons, D. M.
POLYETHER POLYAMINES AS LINKING AGENTS FOR PARTICLE REAGENTS USEFUL IN IMMUNOASSAYS

US 4581337, Apr. 8, 1986
Assignee: E. I. Du Pont de Nemours and Co.

Particle reagents, having polyether polyamine linking groups, for use in turbidimetric immunoassays, are described. These linking agents permit ready covalent attachment of compounds of biological interest to polymer particles.

Gadow, A. E., and Wood, W. G.
LUMINESCENCE IMMUNOASSAY FOR HAPTENS AND CHEMILUMINESCENCE LABELED HAPTEN CONJUGATES
US 4645646, Feb. 24, 1987
Assignee: Henning Berlin GmbH Chemie-und Pharmawerk

Luminescence immunoassays for haptens can be made more sensitive by using a luminescence labeled hapten conjugate. This conjugate contains as the linkage group, a chain-like polymer having repeating functional groups, bound to groups capable of luminescence and hapten. The antibody used is preferably one which is prepared by the use of another chain-like polymer having the hapten bound to it by a different chemical reaction.

Hinshaw, J. C., Toner, J. L., and Reynolds, G. A.
FLUORESCENT LABELS FOR IMMUNOASSAY
US 4637988, Jan. 20, 1987
Assignee: Eastman Kodak Co.

Stable fluoroscent labels are described consisting of a complex of lanthanide metal and a chelating agent having a nucleus which is a triplet sensitizer with a triplet energy greater than that of lanthanide metal and at least two heteroatom-containing groups that form coordinate complexes with lanthanide metals and a third heteroatom-containing group appended to the triplet sensitizer. Fluorescently labeled physiologically active materials such as labeled antigens, heptens, antibodies, and hormones are also described.

Karmen, A., and Lasky, F. D.
IMMUNOASSAY
US 4600690, July 15, 1986
Assignee: Albert Einstein College of Medicine of Yeshiva University, A division of Yeshiva University

A method for immunoassay and/or competitive binding assay is described where an excess amount of unlabeled antigen is added after the start of the reaction between a labeled ligand, an unlabeled ligand, and an antibody, to saturate or flood the antibody binding sites. This provides increased sensitivity, allowing for more reliable and precise movements of ligands such as antigens.

Kerschensteiner, D.
SOLID-PHASE IMMUNOASSAY SUPPORT AND METHOD OF USE THEREOF
US 4623629, Nov. 18, 1986

A solid-phase immunoassay support is prepared by treating a test tube liner made of a preformed soft gelatin capsule half with a coupling solution made of a fixative, a bifunctional coupling agent and a bioactive protein. The support is particularly applicable to the radioimmunoassay (RIA).

Kirkemo, C. L., and Shipchandler, M. T.
AMINOMETHYLFLUORESCEIN DERIVATIVES
US 4614823, Sep. 30, 1986
Assignee: Abbott Laboratories

A method and reagents are described for determining ligands in biological fluids employing a novel class of tracer compounds as reagents in fluorescence polarization immunoassays.

Kosak, K. M.
BIOLUMINESCENT TRACER COMPOSITION AND METHOD OF USE IN IMMUNOASSAYS
US 4604364, Aug. 5, 1986

A tracer composition is described in which a nonradioactive photon emitter, such as luciferase, is coupled to a ligand, antigen or antibody for use in immunoassays. These photon emitters are coupled to antigens or antibodies using coupling agents such as glutaraldehyde, CNBr, and carbodiimide.

Kricka, L. J., Thorpe, G. H. G. H., and Whitehead, T. P.
ENHANCED LUMINESCENT OR LUMINOMETRIC ASSAY
US 4598044, July 1, 1986
Assignee: National Research Development Corp.

A phenolic compound such as 4-iodophenol, 4-phenylphenol or 2-chloro-4-phenylphenol, is used to enhance the sensitivity of a luminescent reaction carried out in an immunoassay between a peroxidase, an oxidant, and a chemiluminescent, 2,3-dihydro-1,4-phthalazinedione.

Kung, V. T., and Canova, D. E.
LIPOSOME IMMUNOASSAY REAGENT AND METHOD
US 4622294, Nov. 11, 1986

A liposome assay reagent for determination of an analyte in a homogeneous immunoassay is described. The reagent includes a suspension of oligolamellar lipid vesicles containing encapsulated glucose-6-phosphate dehydrogenase (1–10 U/MU mol vesicle lipid), and glucose-6-phosphate

(G6P) (5 m*M*). The encapsulated G6P protects the enzyme against inactivation on preparation, by reverse phase evaporation in the presence of organic solvent, and on storage as an aqueous suspension.

Lau-Hon-Peng, P., Yang, E. K., and Jacobson, H. W.
COATED CHROMIUM DIOXIDE PARTICLES
US 4661408, Apr. 28, 1987
Assignee: E. I. Du Pont de Nemours and Co.

Chromium dioxide has favorable magnetic properties that make it desirable as a solid support in heterogeneous immunoassays. However, to be useful in such assays it must be protected against hydrolytic degradation. This invention provides magnetic particles useful in immunoassays, having a core of chromium dioxide that has a reduced surface, the core coated with silica and further coated with a silane.

Nicoli, D. F., and Elings, V. B.
IMMUNOASSAY USING OPTICAL INTERFERENCE DETECTION
US 4647544, Mar. 3, 1987

A method is described for the optical detection of a binding reaction between a ligand and an antiligand. A pattern is formed by a spatial array of microscopic dimensions of antiligand material, ligand material interacting with the antiligand material to produce a binding reaction. A source of optical radiation is directed to the pattern at a particular incidence angle to produce scattering of the energy from the pattern. An optical detector, located relative to the pattern and aligned with a Bragg scattering angle, is used to detect the strong scattering intensity to produce a signal representative of the binding reaction.

Parham, M., and Warren, W. J.
ASSAY OF PEROXIDASE ENZYME ACTIVITY
US 4596770, June 24, 1986
Assignee: Travenol-Genetech Diagnostics

Aqueous *N*-methyl pyrrolidone is used as a solvent for a substrate containing tetraalkyl benzidine chromogen and a peroxide in determining peroxidase enzyme activity. It provides increased ability of the substrate solution and decreased substrate drift in carrying out enzyme-linked immunosorbent assays.

Rokugawa, K.
SIMULTANEOUS QUANTITATIVE IMMUNOASSAY FOR DIFFERENT ANTIGENS OR ANTIBODIES
US 4623618, Nov. 18, 1986
Assignee: Tokyo Shibaura Denki Kabushiki Kaisha

An immunoassay is described by which several kinds of antigens or antibodies in one test sample may be simultaneously quantified. Several

kinds of microcapsules are first provided one for each kind of antigen or antibody to be quantified. Each of the microcapsules binds a specific antibody or antigen. The microcapsules are comprised of a membrane capable of being lysed by the complement activity, and each kind of microcapsules contains within it a substance that is quantifiable and does not interact with another quantifiable substance contained in other kinds of microcapsules. Different kinds of microcapsules are mixed with a test sample and complement, and the quantifiable substances are released from the microcapsules upon their lysis. The complement activity are then quantified.

Self, C. H.
IMMUNOASSAY WITH AN INCREASING CYCLIC DETECTION SYSTEM
US 4598042, July 1, 1986

An immunoassay is described which uses an antibody or antigen-enzyme conjugate which converts a precursor to a cycling agent used in a cycling detection system. The amount of cycling agent, such as NAD to NADH, is continually increased permitting amplification.

Tokinaga, D., Kobayashi, T., and Imai, K.
IMMUNOASSAY METHOD
US 4628035, Dec. 9, 1986
Assignee: Hitachi, Ltd.

An immunoassay method is described for measuring a concentration of an antigen for a short period of time by immobilizing an antibody over the whole zone of an effective supporting matrix for electrophoresis and fixing an antigen in a sample to be measured by electrophoresis for the antigen-antibody reaction between immobilized antibody and the antigen.

Tsay, Y. G., and Shah, V. D.
KINETIC RADIOIMMUNOASSAY TEST METHOD AND DEVICE
US 4618485, Oct. 21, 1986
Assignee: International Immunoassay Laboratories, Inc.

A radioimmunoassay based on competitive binding is described in which immunoreactions are halted at a time when the rate of change of the quantity of bound radiolabeled analyte of interest is inversely proportional to the concentration of analyte of interest in an unknown sera. This test device has a single calibration curve that is accurate throughout its entire shelf life.

Wang, C. H. J., Stroupe, S. D., and Jolley, M. E.
FLUORESCENT POLARIZATION IMMUNOASSAY UTILIZING SUBSTITUTED TRIAZINYLAMINOFLUORESCEIN AMINOGLYCOSIDES

US 4593089, June 3, 1986
Assignee: Abbott Laboratories

A method is described for determining ligands in biological fluids using a fluorescent polarization immunoassay procedure and to a novel class of tracer compounds. This procedure combines the specificity of an immunoassay with the speed and convenience of fluorescent polarization techniques.

Zuk, R. F., and Litman, D. J.
FLUORESCENT MICROBEAD QUENCHING ASSAY
US 4654300, Mar. 31, 1987
Assignee: Syntex (USA) Inc.

A method for performing immunoassays is described using conjugated fluorescent particles and conjugated catalyst where the particles and catalysts are conjugated to members of a specific binding pair. Bound to the fluorescent particle is a catalyst, usually enzyme, member of a signal producing system. Another catalyst is bound to a specific binding pair member. The catalys-specific binding member conjugate becomes bound to the particle, producing a quenching product that binds to the particle, resulting in a reduction in fluorescence.

ENZYME ELECTRODES AND ENZYMATIC ASSAYS

Fossati, P.
ENZYMATIC UREA ASSAY
US 4608335, Aug. 26, 1986
Assignee: Miles Italiana S.p.A.

A method useful as an enzymatic urea assay is described that is based on the use of urea amidolyase pyruvate kinase, pyruvate oxidase, mono and divalent cations, phosphoenolpyruvate, thyamine pyrophosphate, ATP, bicarbonate, phosphate, a color indicator system and inorganic phosphate, a buffer (pH 6.5 to 9.5), and sodium or potassium ferrocyanide.

Freeman, A., and Tor, R.
ENZYME MEMBRANES FOR ELECTRODES
US 4659665, Apr. 21, 1987
Assignee: Ramot University Authority for Applied Research and Industrial Development Ltd.

A film or membrane containing a biologically active protein is prepared from a polymer substituted with acyl hydrazide groups. The polymer is an acrylamide/methacrylamide copolymer (70/30) and the film or membrane is about 30–100 microns thick. The film or membrane is prepared on an electrode and crosslinked to produce an enzyme electrode.

Gallacher, J. J.
STABILIZATION OF INDICATORS FOR DETECTING ENZYME ACTIVITY
US 4615972, Oct. 7, 1986
Assignee: Immuno Concepts, Inc.

A stabilized indicator powder is described for use in assays to detect the presence of peroxidase where the indicator is stabilized by being combined with a water soluble polymer. As a dry powder, the indicator retains its reactivity for at least several months. The powder readily dissolves in an aqueous medium and as a solution retains its reactivity for a period of weeks, even in the presence of peroxide.

Higgins, I. J., Hill, H. A. O., and Plotkin, E. V.
SENSOR FOR COMPONENTS OF A LIQUID MIXTURE
US 4545382, Oct. 8, 1985
Assignee: Genetics International, Inc.

A sensor electrode is described which is comprised of an electrically conductive material having at least one external surface, an enzyme catalytic for a component in a mixture, and a mediator compound that transfers electrons from the enzyme to the electrode when catalytic activity takes place. It can be used as an in vivo glucose sensor either with a silver electrode coated with glucose oxidase and a polyviologen as the mediators, or with a particulate carbon electrode, glucose oxidase, and chloranil or fluoranil as mediator. In another system, bacterial glucose dehydrogenase or glucose oxidase is used and/or ferrocene or a ferrocene derivative as the mediators to give electrodes with improved linearity, speed of response, and insensitivity to oxygen.

Hill, H. A. O., Page, D. J., Walton, N. J., and Whitford, D.
SURFACE-MODIFIED ELECTRODE AND ITS USE IN A BIOELECTROCHEMICAL PROCESS
US 4655885, Apr. 7, 1987
Assignee: National Research Development Corp.

A bioelectrochemical process is described in which the electrons are transferred directly, without use of a mediator between an electrode and an electroactive biological material in either direction. Rapid electron transfer has previously been achieved between an electrode and the positively charged protein horse-heart cytochrome c by adding a surface-modifier such as 4,4′-bipyridyl. It has now been found possible to promote electron transfer to either a positively or a negatively charged protein using the same surface-modifier for either job. This modifier is a pyridine substituted in the 2 or 3 positions with ethyl or methyl groups and in the four position with a (methylene)hydrazinecarbothioamide group.

Itoh, N., and Matsunaga, K.
METHOD AND COMPOSITION FOR DETERMINATION OF GLYCEROL
US 4636465, Jan. 13, 1987
Assignee: Amano Pharmaceutical Co., Ltd.

A method is described for determining glycerol using a reagent system comprised of glycerol dehydrogenase and a pyridine nucleotide coenzyme. An enzyme such as triokinase and dihydroxyacetone kinase is incorporated into the reagent system to eliminate the formed dihydroxyacetone or D-glyceraldehyde in the assay system.

Karasawa, Y., and Takata, Y.
MALTOSE SENSOR
US 4547280, Oct. 15, 1985
Assignee: Hitachi, Ltd.

A method is described for assaying maltose to quantitatively determine amylase. An enzyme membrane having immobilized alpha-glucosidase and glucose oxidase is used with a hydrogen peroxide electrode and a palladium cathode.

Kusakabe, H., Yamauchi, H., and Midorikawa, Y.
USE OF NOVEL L-GLUTAMIC ACID OXIDASE
US 4614714, Sep. 30, 1986
Assignee: Yamasa Shoyu Kabushiki Kaisha

An analytical assay method is described for L-glutamic acid in a sample using L-glutamic acid oxidase, which forms alpha-ketoglutaric acid, ammonia, and hydrogen peroxide. A kit for this analysis uses a biosensor employing this enzyme.

Malloy, T. P., and DeFilippi, L. J.
SURFACE-MODIFIED ELECTRODES
US 4581336, Apr. 8, 1986
Assignee: UOP Inc.

Surface-modified electrodes comprised of an enzyme immobilized on a support are described that may be used in electrochemical cells for production of electrical energy. The support consists of a coating of carbonaceous pyropolymer possessing carbon and hydrogen atoms composited on a high surface area refractory inorganic oxide such that the carbonaceous pyropolymer monolayer coating replicates the surface area and macropore volume of inorganic oxide. The coated support is then impregnated with water-soluble polyamine followed by a solution of excess bifunctional reagent to form a copolymer that provides pendant bonding sites. The treated support is then contacted with an excess of an enzyme to immobilize it. The immobilized enzyme will act as a working electrode in the

presence of a predetermined substrate such as glucose to provide electrical energy.

Kiffer, R.
ENZYME ELECTRODE AND METHOD FOR DEXTRAN ANALYSIS
US 4552840, Nov. 12, 1985
Assignee: California and Hawaiian Sugar Co.

A method for the potentiometric determination of a dextran solution is provided where the dextran is enzymatically hydrolyzed to glucose, the glucose is oxidized to form hydrogen peroxide, and the hydrogen peroxide is measured utilizing a redox electrode. A novel electrode having a plurality of enzyme impregnated layers is provided for converting the dextran to glucose.

Rokugawa, K.
APPARATUS FOR MEASURING VELOCITY OF ENZYME REACTION
US 4621059, Nov. 4, 1986
Assignee: Kabushiki Kaisha Toshiba

An enzyme is immobilized to a capillary column, and a solution containing a chemical substance and a luminescent substance flows through the column. A bundle of optical fibers are arranged along the longitudinal direction of the column, and the luminescence in the column is exposed to a photodiode array through the fibers. The distribution of the luminescent intensity along the longitudinal direction of the column is detected by the photodiode array. Thus, the rate of increase in the product produced by the enzyme reaction can be measured while the solution is flowing through the column, thereby producing the enzyme activity by both end assay and rate assay.

Seago, J. L.
PERFLUOROSULFONIC ACID POLYMER-COATED INDICATOR ELECTRODES
US 4604182, Aug. 5, 1986
Assignee: E. I. Du Pont de Nemours and Co.

A method is described for the amperometric determination of the concentration of a constituent, in a biological sample, which is a substrate for an oxidase enzyme. The substrate is reacted with the enzyme, consuming oxygen and producing hydrogen peroxide, and the oxygen consumption or hydrogen peroxide production is measured with an inert metal indicator electrode coated with a thin film of a perfluorosulfonic acid polymer. The reaction chamber provides means for contacting the sample with the enzyme and means for making amperometric measurements utilizing inert metal indicator electrodes coated with a thin film of a perfluorosulfonic acid polymer.

Watanabe, H., Mitsuhida, N., Andoh, M., and Matsumoto, H.
METHOD FOR DETERMINATION OF HYDROGEN PEROXIDE BY CHEMILUMINESCENCE ANALYSIS
US 4647532, Mar. 3, 1987
Assignee: Toyo Boseki Kabushiki Kaisha

A method is described for determination of hydrogen peroxide by chemiluminescence analysis. A sample containing hydrogen peroxide with an oxidizable nonfluorescent substance in the presence of an oxidizing catalyst to convert the non-fluorescent substance to a fluorescent substance. The fluorescent substance is reacted with an oxalic acid di-ester and hydrogen peroxide in the presence of an inhibitor which inactivates the oxidizing catalyst. The amount of light emission produced is then measured to determine the amount of hydrogen peroxide contained in the sample.

Wegfahrt, P. F., Jr.
ASSAY SYSTEMS BASED ON MAGNESIUM-RESPONSIVE ENZYMES
US 4657854, Apr. 17, 1987
Assignee: Modrovich, I. E.

An assay method is provided for determining the concentration of magnesium ions in biological fluids. The method introduces a sample of biological fluid to a magnesium-free assay solution containing a reactant and an enzyme capable of catalyzing a reaction. The rate of reaction is dependent on and correlatable to the concentration of magnesium that is present.

LITERATURE

This section surveys the literature in the area of immunological, enzymatic and hybridization probe assays published from January 1986–May 1987. This section is not intended to be all encompassing and lists only some of the major review articles that appeared during this time period.

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