Basic Technology and New Products at HORIBA Biotechnology

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Abstract

HORIBA Biotechnology Co., Ltd. (HBT) is a venture company established in June 2000, with the aim of developing the analytical systems which are rapid and highly sensitive for environmental hazardous chemical substances such as residual pesticides and dioxins by using frontier biotechnologies. This report introduces the background of our company establishment, the assay kits using enzyme-linked immunosorbent assay (ELISA) which is one of the immunochemical analyses and the MPR-01 micro plate reader, and our future direction.

1 Introduction

HORIBA Biotechnology Co., Ltd. (HBT) was established in June 2000, for the purpose of commercially developing the seeds of technology which have been developed by HORIBA.

First of all, we started to fuse the results of research by Professor Hideo Ohkawa at the Kobe University Reserch Center for Environmental Genomics to HORIBA's knowledge of instrumental analysis. Professor Ohkawa has made many substantial and important advances the field of detecting ultramicro substances by using antibodies which selectively react with a specific substance. HORIBA has commercialized many unique measurement devices using semiconductor sensors. Our company aims to develop and sell unique measurement devices for the environmental field by making maximum use of both seeds and basic technologies.

Just after the company's establishment, HBT got Professor Ohkawa as a director. On the other hand, it joined a national research development project which had at that time been promoted by the Ministry of International Trade and Industry, called the "Eco monitoring project." The new company started "Development for highly sensitive detection and measurement of chemical substances in the environment by using biological functions." In the year 2001, HBT was transferred the research results about the immunochemical analyses for residual pesticides, including the patent and clones which HORIBA purchased from Environmental Immuno-Chemical Technology Co., Ltd. established with the support of the Japan Key Technology Center (foundation). HBT launched out as the just venture company which embarked from the university.

In 2002, the main office (Fig.1) was relocated from HORIBA and a research/production building was built to put the project in full swing. Moreover we joined a new national project promoted by the Kansai Bureau of Economy, Trade and Industry, and expanded our complement of equipment and devices as well as enhanced our research and development environment by hiring many researchers and technical experts in the field of biotechnology and measurement (Fig.2a, b). In 2003, in addition to biotechnology (BT), we have kicked off work in the merged field of nanotechnology (NT) and information technology (IT).

Fig.1 Main Office Premise

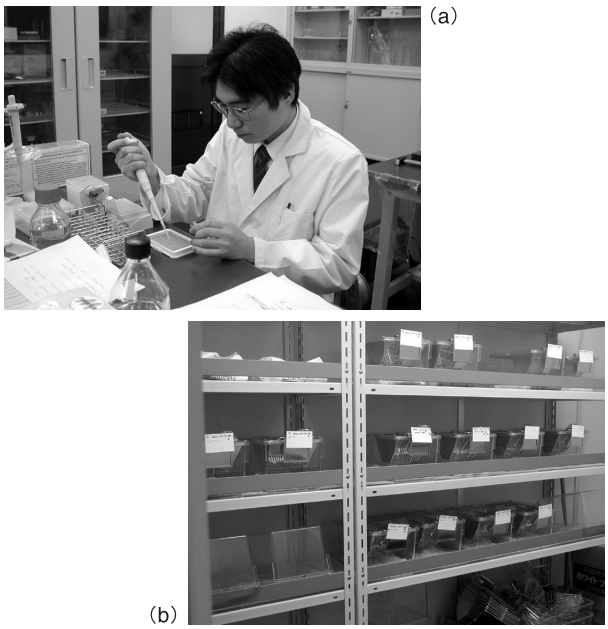

Fig.2 Examples of Research and Development Environment
(a) Basic Experiment (b) Laboratory Mouse Vivarium

2 Determination Ultramicro Chemical Substances by the Immunochemical Analysis

In blood tests conducted as part of the periodic health checks at schools and companies, hormones, enzymes, or proteins are analyzed quantitatively. Most of these tests are using the examination kits which are based on the immunochemical analysis.

Usually, animals protect their bodies using the lymph cell's immune system when viruses, bacteria, or snake venom invades their bodies. Lymph cells produce an antibody (a kind of protein) which selectively bonds to foreign material (the antigen) and this antibody sticks to the antigen to deactivate it. This is called the humoral immune system. Then the antigen bonded with the antibody is decomposed by a phagocyte called macrophage (one of the leucocytes) and eliminated from the living body. This is called the cellular immune system. As mentioned above, our bodies are protected from foreign material all the time by the humoral and cellular immune systems. The method for analyzing ultramicro chemical substances using these immune mechanisms is called the immunochemical analysis.

In the past, ultramicro chemical substances such as residual pesticides have been analyzed by such methods as high performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS). However, the equipment used in these methods must be operated by professional technicians and requires several days or even up to two weeks to obtain the analysis results because it is so complicated.

Bovine spongiform encephalopathy (BSE) or "mad cow disease," which caused fear in Europe and Japan in recent years, is said to cause destruction of the brain because of the presence of small proteins called prions. In the effort to create a screening test for mad cow disease, recently the immunochemical analysis has attracted attention because massive amounts of samples can be tested easily and at low cost.

HBT is now developing assay kits using the Enzyme Linked Immuno-Sorbent Assay (ELISA), which is one of the immunochemical analyses, for analyzing residual pesticides in agricultural products, food, soil, or river water.

3 Preparation of Antigens and Antibodies

For immunochemical analysis, it is the greatest theme to produce highly sensitive, selective, easily-handled, and low-cost antigens and antibodies, selected for the target chemical substance to be analyzed. HBT is preparing the antigens and antibodies using the latest biotechnology, and is manufacturing the assay kits using these antigens and antibodies.

3.1 Chemical Synthesis of Hapten Antigen

Most of the agrichemicals used currently are chemical syntheses, with molecular weights from 100 to 300 and it is difficult to produce an antibody by using the antibody-producing cell (lymph cell). Therefore by chemically synthesizing the hapten antigen, which conjugates small molecular weight agrichemicals to the surface of large molecular weight proteins such as albumin in blood, the antibody-producing cell recognizes the agrichemicals as foreign material and produces an antibody.

3.2 Monoclonal Antibody-producing Hybridoma

If it is possible to have a cell with the ability to produce antibodies which grows immortally as do cancer cells, it will be possible to produce the same antibody immortally, even outside the living body. It was the German immunologist, Georges J.F. Kohler and César Milsein, who made the idea become reality. They fused the normal lymph cell and the myeloma to produce a hybrid cell (hybridoma) and used this hybridoma to obtain the monoclonal antibody in 1975.

HBT has established a technique for preparing the cell line (hybridoma), which produces monoclonal antibodies from mice based on this technique, and now we have established the system needed to accept requests of entrusted development from clients.

Fig.3 shows the procedure for producing the monoclonal antibody.

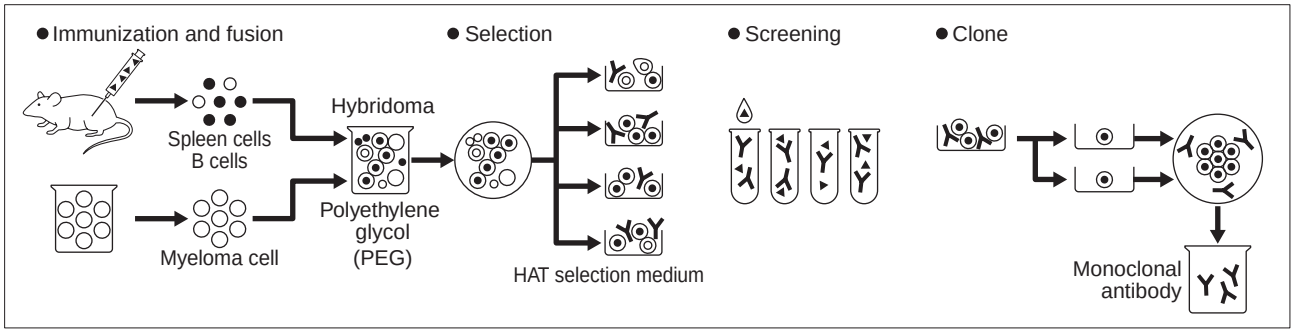

Fig.3 Procedure for Producing the Monoclonal Antibody

3.3 Producing Antibody by Gene Recombination

The technology for producing antibody by gene recombinating technique is a method for producing the objective antibody massively and at low cost by separating the antibody-producing genes, which are obtained from the antibody-producing cell line stated above, implanting the obtained gene into Escherichia coli, and multiplying the Escherichia coli in a culture media. HBT is putting this technology to practical use in its Eco-monitoring project. Fig.4 shows the cloning process of the anti-bisphenol A monoclonal antibody. Bisphenol A is suspected to be an environmental hormone mimic (endocrine disrupting chemical), found in many parts of the world.

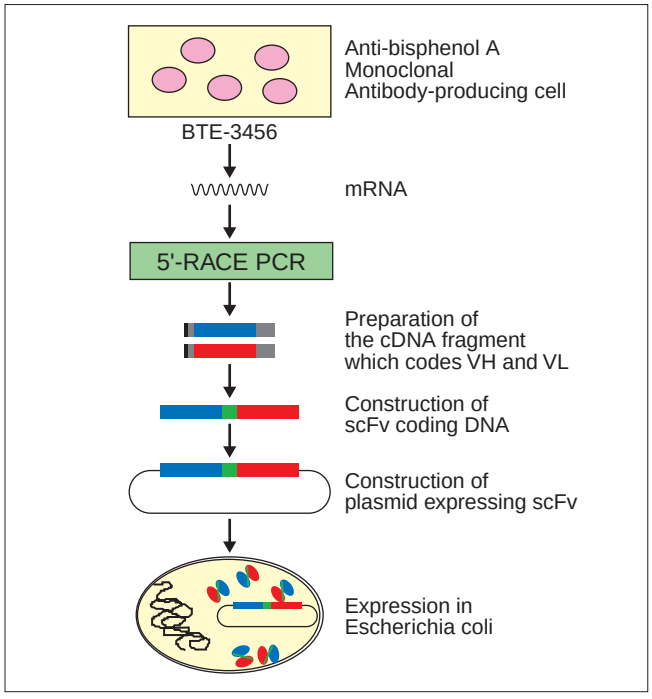

Fig.4 Cloning Procedure of the Variable Regions of the Anti-bisphenol A Monoclonal Antibody

4 Assay Kit for ELISA Analysis

The assay kit for ELISA analysis consists of a microplate with 96 wells, and several kinds of physiologically active substances such as secondary antibody reagent, coloring reagent, stop reagent for coloring, and standard reagent for calibration curve. Fig.5 shows the kit and Table 1 lists its main specifications.

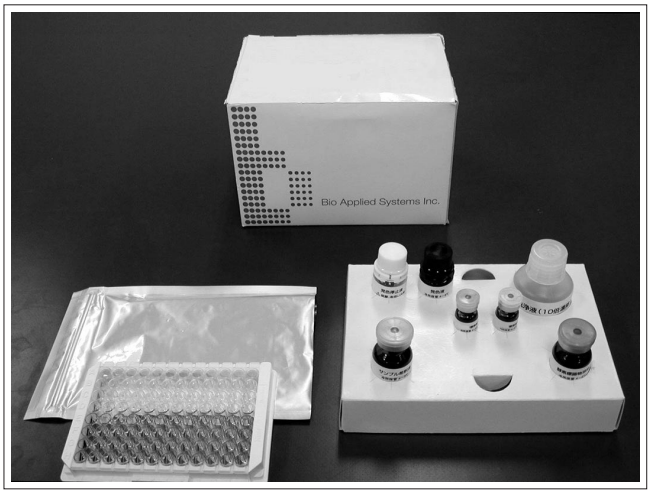

Fig.5 Assay Kit for ELISA Analysis

Table 1 Main Specifications of the ELISA Analysis Assay Kit (for measuring the agricultural chemical acetamiprid)

Name	capacity	form	quantity
Antibody plate	8 wells × 12 rows	dry	1 plate (96 wells)
Acetamiprid Standard reagent L (0.3 ppb)	1 mL	freeze dried	1 vial
Acetamiprid Standard reagent H (4 ppb)	1 mL	freeze dried	1 vial
Enzyme-labeled substance reagent	5 mL	freeze dried	2 vials
Washing reagent (10 times condensation)	50 mL	liquid	1 vial
Coloring stop reagent	13 mL	liquid	1 vial
Coloring reagent	13 mL	liquid	1 vial

4.1 Measurement Process

HBT's assay kit for immunochemical analysis introduces the direct competitive ELISA method. The measurement procedure is as follows. Fig.6 shows the measurement flow of the direct competitive ELISA method.

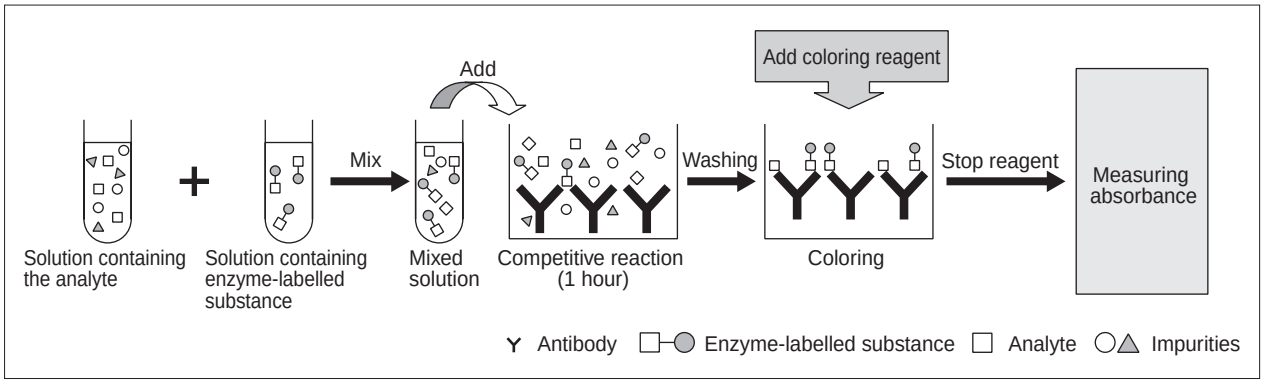

Fig.6 Measurement Procedure in Direct Competitive ELISA Method

(1) Immobilization of Antibody

Immobilize the antibody, which specifically combines with the analyte, at the bottom of the wells on the 96-wells micro plate.

(2) Labeling the Analyte Using Enzyme

The carboxylic acid derivative of the analyte is synthesized and conbined with the enzyme (horse radish peroxidase) lysine residue by the covalent bond.

(3) Competitive Reaction

Mix the solution containing the analyte and the solution containing the enzyme-labelled substance in the test tube. Add this mixed solution into the well where the antibody is already immobilized and make it react competitively. After approximately 1 hour of reaction, remove the antibody and non-reacted substance by the washing.

(4) Color Development Reaction

Add the color development reagent for enzyme into the well on the washed plate. If the enzyme-labelled substance reacts with the immobilized antibody, the color reagent will be colored by the enzyme reaction. If the concentration of the analyte is high, it will not develop color. However if the concentration is low, the degree of color development will increase.

(5) Result of Measuring the Target Substance

The degree of color development on the coloring reaction changes according to the concentration of the analyte. Therefore the concentration of the analyte in the sample can be calculated by making the standard curve of these relationship.

However, steps (1) and (2) are done with the plate in the kit in advance, so only (3) through (5) should be performed.

4.2 Micro Plate Reader

HBT created the MPR-01 micro plate reader (Fig.7), which is suitable for the immunochemical measurement reagent kit. This is a desktop device which can measure, calculate, and display absorbance accurately and has the following features.

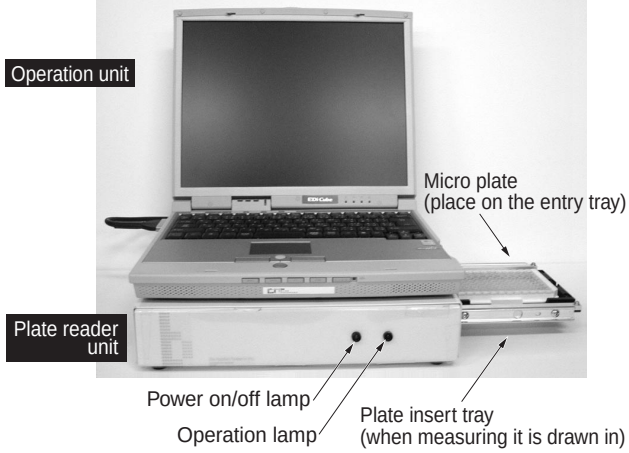

Fig.7 MPR-01 Micro Plate Reader

(1) It can measure a wide range of concentration (0 to 2.9 Abs) using the light-emitting diode.

(2) It measures at high speed, less than 10 seconds per micro plate.

(3) It is easy to convert and use the measurement results because they are processed with a general-purpose spreadsheet software.

(4) A thin, compact shape, which does not occupy much space even in a small laboratory.

Table 2 shows the main specification of the MPR-01.

Table 2 Main Specifications of the MPR-01

[Plate reader unit]

Photometry style	8 line-parallel light route Vertical lighting style
Measurement range	0 to 2.9Abs
Measurement wavelength	450nm
Measurement mode	Single or double wave length Portrait or landscape scan
Available measurement plat	96-wells micro plate
Measurement time	Less than 10 seconds/plate
Light source	Light-emitting diode
External connection	D-sub (37 pins) 1 port
Dimensions/weight	300(W) × 300(D) × 65(H) mm Approx. 7 kg

[Operation unit]

Operation unit	Notebook PC
External connection	A/D transmit PC card + contact button (D-sub (37 pins))

5 Conclusion

Remarkable developments in scientific technology lead to many conveniences. On the other hand, it is suggested that ultramicro chemical substances such as residual agricultural pesticides, endocrine-disrupting chemicals (environmental hormones) in the water, and dioxin in the soil are threatening the basis of ecosystem. In order to identify and eliminate these dangers, it is necessary to have a system for analyzing ultramicro chemical substances rapidly, easily and with a high sensitivity.

We are sure that the immunochemical assay, which HBT is now developing, is the best method to meet these prescribed needs.

It is said that biotechnology is the most important core technology to bring benefits to mankind in the twenty-first century. However at this point, science has used only a small number of the functions found in living bodies. HBT would like to contribute to mankind's true happiness by applying the latest biotechnology and information technology, based on the analytical technology which HORIBA has been developing for more than 50 years.

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