# $DioQuicker \ ({\rm Dioxin\ ELISA\ Kit})$

These days, levels of dioxin in the environment are measured by means of instrumental analysis methods with a high-resolution gas chromatograph mass spectrometer (HRGC/HRMS), which is generally an expensive analytical instrument. The instrumental analysis methods, however, have the following shortcomings.

· It takes several weeks to get the measurement results.

## Introduction

- The measurement costs a great deal of money.
- For the reasons above, it is difficult to perform "daily measurement" for the purpose of preventing dioxin and dioxin-like compounds from being generated.

Therefore, simple methods based on ELISA using antibodies have been developed as simpler methods than the instrumental analysis methods. But it was considered to be difficult to evaluate toxicity equivalency quantity by means of the conventional ELISA methods, because they employed antibodies that specifically reacted to 2,3,7,8 tetrachloro-dibenzo-p-dioxin (2,3,7,8-TeCDD), which is highly toxic among dioxin and dioxin-like compounds, but exists in minute quantities within environmental samples, and therefore did not react so well to the other dioxin isomers, which highly correlate with <sup>note)</sup> dioxin Toxicity Equivalency Quantity (TEQ) in the sample.

This kit is a dioxin measurement kit based on a competitive enzymatic immunoassay method using antibodies that identify pentachlorinated and hexachlorinated dibenzofuran, which highly correlate with dioxin Toxicity Equivalency Quantity (TEQ).

By using this kit, you can measure dioxin quantity in an environmental sample simply and quickly. Since it also enables the measurement of a number of samples at one time simply and at a lower cost, it is most suitable for screening multiple samples.



## **New Ordinance**

Ministerial Ordinance Partially Amending the Enforcement Regulations of the Law Concerning Special Measures against Dioxins is promulgated in December 2004.

The amendment authorizes simplified measurement methods by bioassay as methods for assessing dioxins in emission gas, dust and cinders from waste incinerators in certain cases.

These simplified measurement methods are defined as:

- a method utilizing feature of dioxins which bond with aryl hydrocarbon
- a method utilizing antigen-antibody reaction of dioxins.

Concrete measuring methods is established by the Minister of the Environment, after technical evaluation made by the Ministry of the Environment (MoE).

DioQuicker( dioxin simple measuring method of our company) is approved one of concrete measuring methods by the Ministry of the Environment.

## **Adaptation limit in Odinance**

1)Measurement of emission gas (waste incinerators with incineration capacity less than 2,000 kg/hour) in the designated facilities under the Law.

2)Measurement of dust, etc. (waste incinerators with incineration capacity no limitation) in the designated facilities under the Law.

3) Verification of treatment standards for dust, etc. in waste incinerators under the Law.

## **Characteristics**

#### 1. Measure multi-samples quickly

This kit employs 96 Well Microplates, which enables multi-sample measurements at one time. Furthermore, the measurement time is as short as about 4 hours.

#### 2. High correlation with the HRGC/HRMS method

Monoclonal antibodies, which highly react to dioxin isomers that contribute to dioxin Toxicity Equivalency Quantity (TEQ) in environmental samples were developed. Therefore, this kit has a high correlation with the instrumental analysis methods (the official method) (effluent gas  $R^2$ =0.99, fly ash  $R^2$ =0.94), and so enables sufficient measurement of the levels for each environmental sample within the range of effluent control.

#### 3. Safety while working

Since this kit employs chlorophenol derivatives as reference materials for creating the analytical curve, it is not necessary to use highly toxic dioxin reference materials. Therefore, safety while working is better than with conventional methods.

#### 4. No extra equipment is needed for storage

This kit can be stored in a refrigerator (2–8 °C). Therefore, no extra equipment is needed for handling or storing this kit.

## **Measuring Principle**

- 1.Add the sample you want to measure and anti-dioxin monoclonal antibody to the anti-dioxin antibody and responsive specific compound bound together on the plastic plate, and then let them react at 4 °C for 1 hour.
- 2.If the dioxin is large in quantity, it becomes difficult to bind with the compound on the plate, because a greater quantity of antibody grows combined with the dioxin according to its quantity.
- 3. Therefore, the less the quantity of antibody is left combined on the plate, the more dioxin proves to be contained in the sample. Conversely, a greater quantity of antibody found connected on the plastic plate indicates that less quantity of dioxin is contained in the sample.
- 4.The quantity of antibody can be quantified by the degree of color that occurs when secondary antibody labeled with enzyme (horseradish peroxidase; HRP) is added to the compound on the plate for reaction. And also, based on the strength of coloring, the dioxin level will be quantified. And, at that moment, measure chlorophenol derivatives at the same time, whose concentrations are already known, as reference materials for creating the analytical curve, and you can calculate the dioxin level as a converted value to the chlorophenol derivatives. Next, calculate the dioxin Toxicity Equivalency Quantity based on the TEQ and regression formula prepared in advance.