Micro Bioactivity Analyzer

AMIS-101

April 2013

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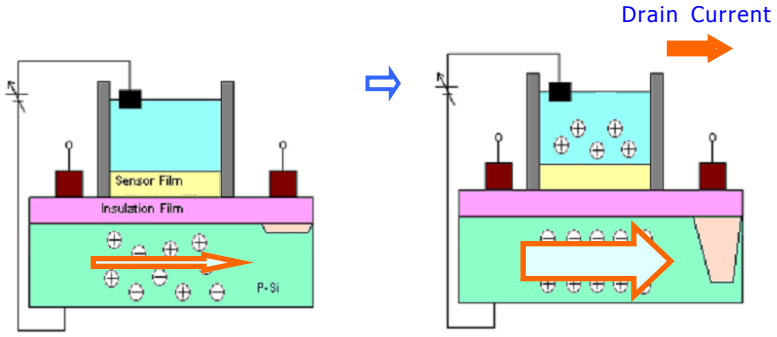
1. Measuring principle of AMIS-101 (Direct conversion of chemical reaction to electronic signal. Labeling and coloring process free)

In the drawing below we show the AMIS (Accumulation Method Ion Sensor) reaction cell (blue box) sitting on top of a sensing membrane (yellow layer). Reagents are combined in aqueous solutions or suspensions which are placed in this cell. As the number of protons in the reaction cell changes over the course of a reaction, the electron polarization in the sensing layer is altered, leading to a current change through the semiconductor.



Polarization in semiconductor caused by chemical ions

Chemical ions cause the polarization in semi-conductor, which change the current volume flowing through the gate.

<Items AMIS-101 can measure>

In this way, the AMIS can measure chemical and biochemical activity in an aqueous solution or suspension.

Including but not limited to.:
Enzyme reactions
Protein metabolic reactions
Bacteria metabolic reactions
General chemical reactions

AMIS-101 makes measurement simple and quick, eliminating colouring / labelling processes, by transferring the reaction directly to an electronic signal.

In addition, solution color and turbidity do not affect results. Both of these advantages allow users to see reactions that may be difficult to visualize using optical approaches such as spectrophotometry.

<Items difficult to measure as it is by AMIS-101>

The device will not be able to measure reactions that do not alter proton levels in the solution. Such reactions can include electric charge changes and structural changes.

For example:

- Aggregation and dispersion of protein
- Structure change of protein
- Antigen-antibody reaction
- Interactive analysis between gene & gene and gene & protein

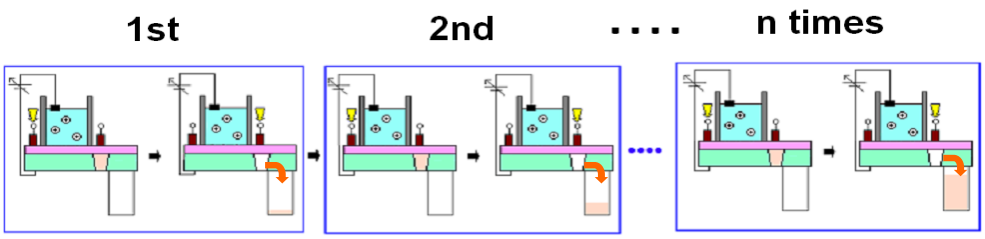
For these kinds of processes, the user may need secondary reactions to create the required proton charges.

2. Various measurements with one sensor

The AMIS sensor is not an ion selective sensor. The same sensor can be used to measure various chemical and biochemical reactions. In each case, different reagents will be selected for different targets. (Contact us for our Data Book.)

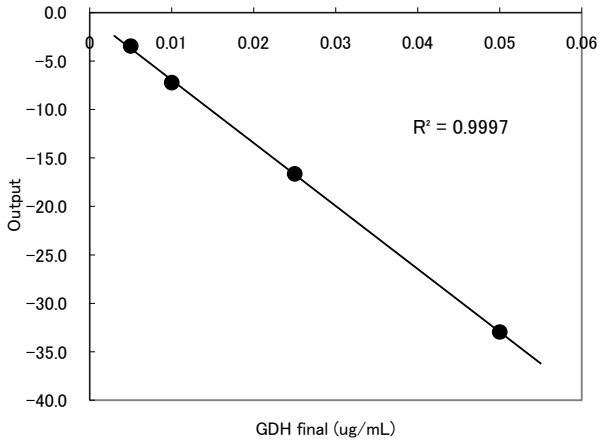
3. High sensitivity

The AMIS sensor consists of CMOS device that stores and accumulates the signal from the sensing layer as an electronic charge. This allows the AMIS to amplify the sensor signal, improving the signal-to-noise ratio and enabling highly sensitive measurements.



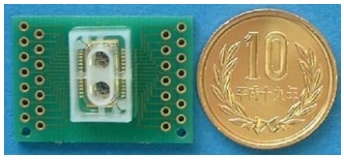
Signals measured per measurement are stored inside the sensor for amplification to improve S/N ratio.

(Example) The detectable limit of Glucosedehydrogenase (Enzyme) in Glucose (substrate) solution (5mg/ml) was 5ng/ml (Approximately 50 fM).



4. Micro volume measurement

The AMIS uses a 20 µl of reaction cell. This allows users to achieve results with minimal amounts of reagent.



Example: The Glucose-Glucosedhydrogenase reaction mentioned above requires only 5ng/ml x 0.02 ml = 0.1ng (Approximately 1 femto mole) of Glucosedehydrogenase.

5. Real time detection

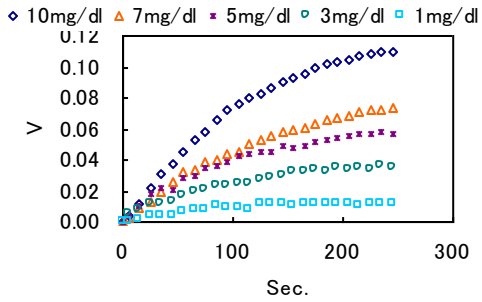
The AMIS system detects reactions in real time and enables quantitative measurement in about 5 minutes.

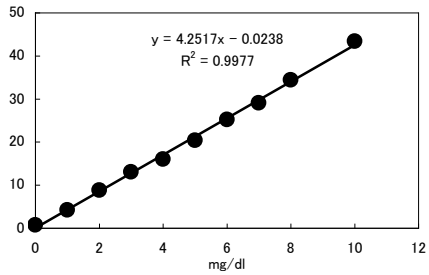
Ex.: Creatinine detection with Creatinine deiminase (enzyme)

AMIS system can detect Creatinine with a single enzyme reaction. No coloring process is required.

$$\text{Creatinine} + H_2O + H^+ \xrightarrow{\text{Creatinine Deiminase}} \text{N-Methylhydrantoin} + NH_4^+$$

Enzyme solution（0.1mg/ml）: 18 ul（Enzyme 1.8 ug）×2 (sample + control)
Substrate : 2ul



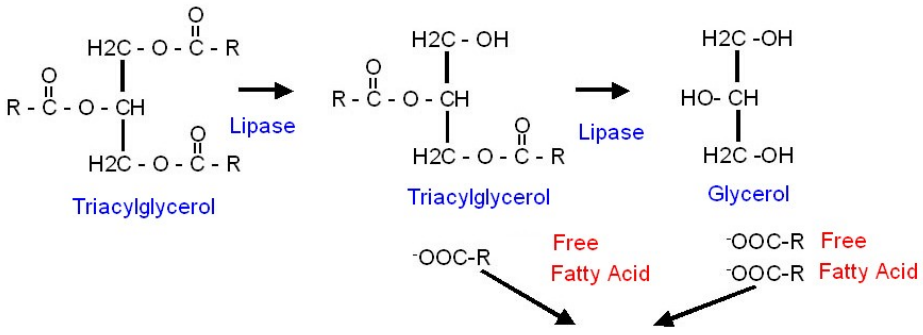


6. Application Examples

6-1 Lipase Activity

Lipase is an important enzyme for clinical and industrial purposes. We expect to finish development of a simple and effective measuring method for this enzyme. AMIS system can detect free fatty acids produced by the enzyme reaction effectively, leading to the development of the system for the quantitative analysis of lipase reaction which is difficult to follow by spectrophotometry.

[Mechanism]



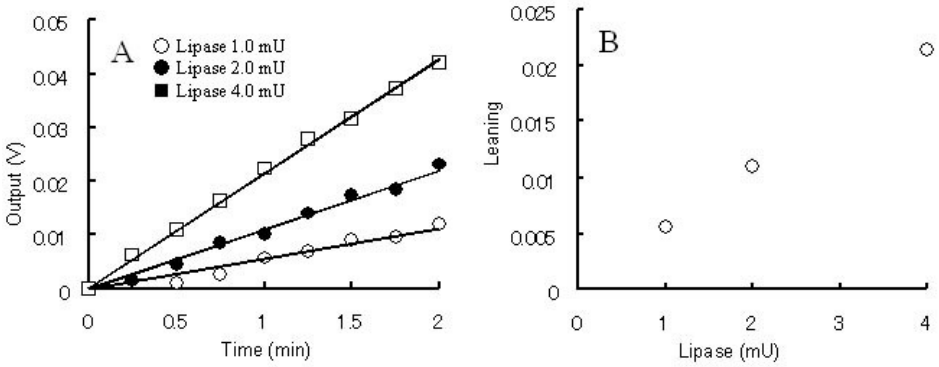
Activity Measurement by AMIS Sensor

[Reagents]

Enzyme: Lipoprotein Lipase (TOYOBO LPL311)

Substrate: Olive oil 3mg/ml in Buffer

Buffer: 2mM Tris-HCl pH8.0 20mM NaCl



Time course of Enzyme Reaction (A)

Enzyme amount vs Output (B)

6-2 Galactose and Lactose detection by measuring Galactose

Galactose in diary products is a physiologically important sugar. However, excess concentration in blood may cause serious damage in brain and/or liver, and so Galactose levels in foods needs to be well controlled. The AMIS system supports a simple and highly sensitive Galactose measuring system.

6-2-1 Galactose

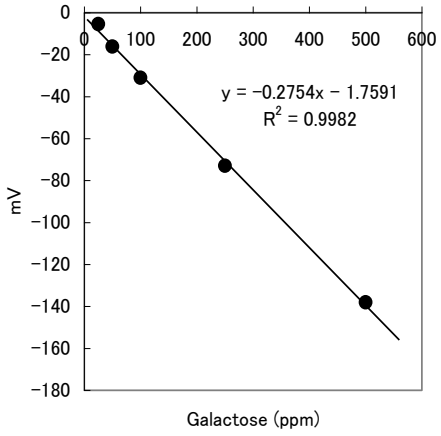
[Reagents]

Enzyme: Galactosedehydrogenase (SIGMA)

Coenzyme: NAD

Substrate: Galactose (Nacalai Tesque)

Buffer: 1mM KPBS (pH7.0)



Galactose concentration vs Output

6-2-2 Lactose

Lactose is produced by degradation of Lactose in the body, and the amount of Lactose in foods should be controlled as well as Galactose.

AMIS system can measure the Lactose by detecting Galactose.

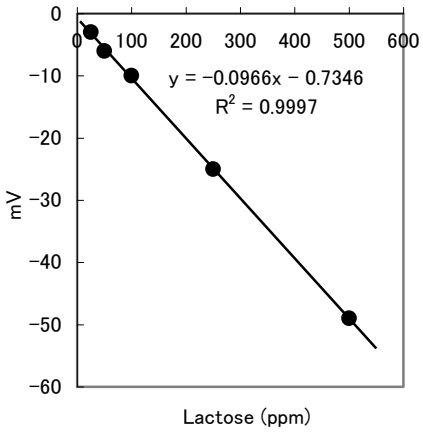
[Reagents]

Enzyme: Beta-Galactosidase (TOYOBO) + Galactosedehydrogenase (SIGAM)

Coenzyme: NAD

Substrate: Lactose (Nacalai Tesque)

Buffer: 1mM KPBS (pH7.0)



Lactose concentration vs Output

6-3 Direct measurement of Total Polyphenols in drink product without Pre-treatment

AMIS Sensor can measure the concentration of Total Polyphenols in drink products. The sample is actual drinks obtained from market. Since AMIS system is not affected by the color nor turbidness of the sample, measurement was done directly on the sample without any pretreatment.

6-3-1 Catechin in Japanese tea

[Reagents]

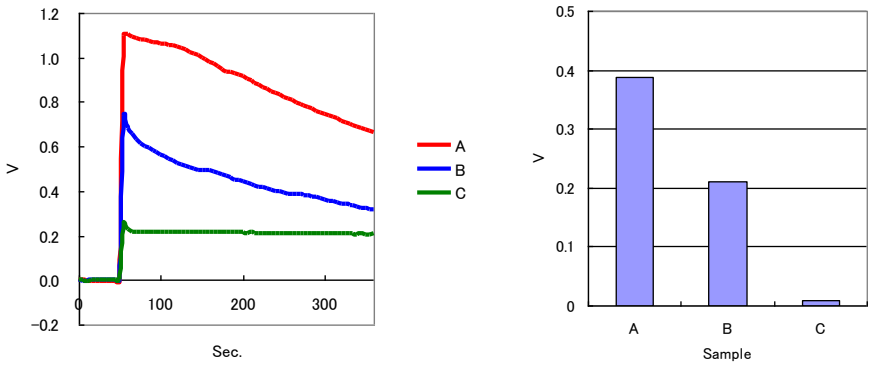
Enzyme: Laccase

Sample: Japanese tea

A: Extra high Catechin contained tea (1.54mg/ml)

B: High Catechin contained tea (0.85mg/ml)

C: Regular tea



Concentration of Catechin vs Output

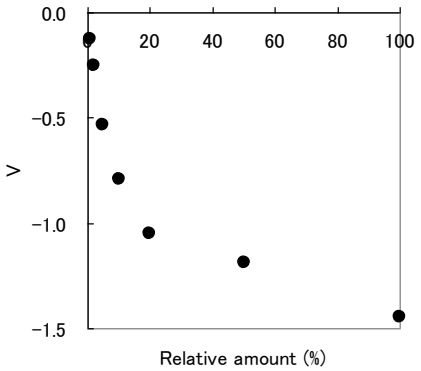
6-3-2 Total Polyphenols in Wine

[Reagents]

Enzyme: Laccase

Sample: Wine diluted with Buffer

Buffer: 1mM Acetate Buffer (pH5.0)



Concentration of Wine (Total Polyphenols) vs Output

6-4 Other Examples

Following is the measured example to show the basic performance of this analyzer, which indicates the possibility of wide range application.

Substrate	Enzyme	Co-enzyme	Substrate Sensitivity (μM)	Enzyme Sensitivity (Aboslute amount in 20μ)	Sensing Object
Glucose	Glucose Oxidase		10μg/ml (55μM)	0.5μg/ml (1ng)	Gluconic acid
Glucose	Glucose Dehydrogenase	NAD	20μg/ml (111μM)		Proton
Glycerol	Glycerol Kinase + Glycerophosphate Oxidase	ATP	1.5μg/ml (16μM)		Peroxide
Ethanol	Alcohol Dehydrogenase	NAD	10μg/ml (217μM)		Proton
Formaldehyde	Formaldehyde Dehydrogenase	NAD	0.3μg/ml (10μM)		Proton + Formic Acid
Acetaldehyde	Aldehydel Dehydrogenase	NAD	5ng/ml (0.1μM)		Proton + Acetic Acid
ATP	Alkaline Phosphatase		5μg/ml (10μM)	0.1unit/ml (0.002unit)	Phosphoric Acid
Urea	Urease		2μg/ml (33μM)		Ammonia
Creatinine	Creatinine Deiminase		1μg/ml (8.8μM)	1μg/ml (2ng)	Ammonis
Olive Oil	Lipoprotein Lipase		100μg/ml		Oleic Acid
Cholesteryl Linoreate	Cholesterol Esterase		200μg/ml (300μM)		Lonleic Acid
DL-BAPNA	Tripsin		60μg/ml		Carboxylicc Acid

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