AB-2970　CLETA-S Quick Manual

Composition (Storage)

CL reagent	1mg--------1 bottle （-20℃）
Substrate solution	20mL-------1 bottle （4℃）
Enzyme solution	8mL--------1 bottle （4℃）
Dilution buffer	8mL--------2 bottles （4℃）

Component

	Main Component
CL reagent	MPEC(powder **1)
Substrate solution	Xanthine
Enzyme solution	Xanthine Oxidase(1.25unit /mL)
Dilution buffer	HEPES

**1：Please make a stock solution by dissolving the 1mg of MPEC powder with 1.78ml ethanol and divide it into small bottle which for each experience, and store it at -80dgreeC.

For working solution, dilute 50μL of stock solution with 150 μL of distilled water.

Summary of Antioxidant Activity Measurment

superoxide is generated from xanthine-xanthine oxidase reaction.

The emission of light is provided by adding a chemiluminescence reagent in generated superoxide.

Function of antioxidant activity is evaluated adding an antioxidant enzyme or an antioxidant material in this reaction.

* CL reagent + Dilution buffer + Substrate solution
・・・・・・・・・・・・Negative Control

* CL reagent + Enzyme solution + Substrate solution
・・・・・・・・・・・・ Positive Control

* CL reagent + Dilution buffer + Substrate solution + Target sample
・・・・・・・・・・・・ Sample (N)

* CL reagent + Enzyme solution + Substrate solution + Target sample
・・・・・・・・・・・・ Sample (P)

the antioxidant activity is calculated in below formula.

Antioxidant activity = 1 – [(sample(P) – sample(N)) / (Positive control – Negative Control)]

Operating Method

Recommended volumes for 1 measurement

(1) Sample (or Blank solution)	10 μL
(2) CL reagent	10 μL
(3) Enzyme solution (or Dilution buffer)	80 μL
(4) Substrate solution	200 μL

{Measurement}

① Prepare mixing solution with CL reagent & Dilution buffer.
Mix 10 μL of CL reagent and 80 μL of dilution buffer per 1 time of measurement.
The solution should be prepared with over turn mixing in appropriate volume for 1 measurement before using.
(ex: prepare mixing solution with measured sample amount × four times and additional two times at a time.)

② Prepare mixing solution with CL reagent & Enzyme solution.
Mix 10 μL of CL reagent and 80 μL of enzyme solution per 1 time of measurement.
The solution should be prepared with over turn mixing in appropriate volume for 1 measurement before using.
(ex: prepare mixing solution with measured sample amount × four times and additional two times at a time.)

③ Inject 10 μL of blank solution into a measuring container. Prepared and diluted sample is contained in the blank solution.

④ Inject 90 μL of prepared ① mixing solution into ③the measuring container.
Set the measuring container to the device, and inject 200 μL of substrate solution.
After measuring of luminescence for 10 sec., the value is considered as [Negative control].

⑤ Inject 10 μL of ③ the blank solution into a new measuring container, and inject 90 μL of ② mixing solution.
Measure it same as process ④, the value is considered as [Positive control].

⑥ Inject 10 μL of a sample into a new measuring container, and inject 90 μL of ① mixing solution.
Measure it same as process ④, the value is considered as [Sample(N)].

⑦ Inject 10 μL of ⑥ the sample into a new measuring container, and inject 90 μL of ② mixing solution.
Measure it same as process ④, the value is considered as [Sample(N)].

⑧ Calculate antioxidant activity with the above mentioned formula.

Reference

1.Shimomura, O., Wu. C., Murai, A., and Nakamura, H. (1998) Evaluation of Imidazopyrazinone-Type Chemiluminescent Superoxide Probes and Their Application to the Measurement of Superoxide Anion Generated by Listeria Monocytogenes: Anal. Biochem 258, 230-235