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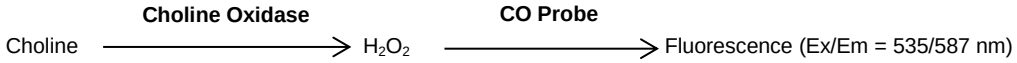
# Choline Oxidase Activity Assay Kit (Fluorometric)

*08/20*

**(Catalog # K2052-100; 100 assays, Store kit at -20°C)**

## I. Introduction:

Choline Oxidase (E.C. 1.1.3.17) belongs to the oxidoreductase family and is present in plants, bacteria, fungi and mammals. It is a catabolic enzyme that catalyzes the chemical reaction of choline and oxygen into betaine glycine and hydrogen peroxide with betaine aldehyde as an intermediate. The enzyme participates in glycine, serine, and threonine metabolism and is commonly used as a tool for the enzymatic determination of acetylcholinesterase, phospholipase D, phosphatidylcholine-specific phospholipase C, sphingomyelinase etc. Betaine glycine is a well known osmoprotectant and can help plants and bacteria to survive dry, cold and salt stresses. As a result, choline oxidase has been engineered genetically to produce transgenic plants such as tomato, rice and tobacco with increased stress tolerance and productivity. Additionally, accumulation of betaine glycine has been observed in bacteria such as *Staphylocccus aureus*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* thereby suggesting that drugs capable of inhibiting choline oxidase activity will have potential anti-bacterial activity. **BioVison's Choline Oxidase Activity Assay Kit** is the first, commercially available kit to determine the activity of choline oxidase in samples. In this assay, choline oxidase oxidizes choline to generate hydrogen peroxide, which then reacts with the probe to generate a strong fluorescent signal measured at Ex/Em = 535/587 nm. The fluorescent signal is directly proportional to the choline oxidase activity in the samples. The assay is simple, fast, reproducible and is high throughput adaptable. The kit can detect as low as 0.2 mU of choline oxidase activity under assay conditions.




## II. Application:

- Measurement of choline oxidase activity

## III. Sample Types:

- Purified protein
- Bacterial cell lysates

## IV. Kit Contents:

| Components | K2052-100 | Cap Code | Part Number |
|---|---|---|---|
| CO Assay Buffer | 25 ml | NM | K2052-100-1 |
| Choline | 1 vial | Orange | K2052-100-2 |
| CO Probe | 200 µl | Red | K2052-100-3 |
| CO Developer | 1 vial | Green | K2052-100-4 |
| $H_2O_2$ Standard | 100 µl | Yellow | K2052-100-5 |
| Choline Oxidase | 1 vial | Blue | K2052-100-6 |

## V. User Supplied Reagents and Equipment:

- Sonicator
- 96-well white, flat-bottom fluorescent plate
- Multi-well spectrophotometer
- 50 mM Phosphate buffer, pH 7.2
- PBS
- 10 mM EDTA

## VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge all small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **CO Assay Buffer:** Ready to use as supplied. Warm the bottle to room temperature (RT) before use. Store at 4°C.
- **Choline:** Reconstitute the vial in 1.1 ml of $dH_2O$. Choline solution is stable for two months at -20°C.
- **CO Probe:** Bring to RT before use. Stable for two months at -20°C.
- **CO Developer:** Reconstitute the vial in 220 µl of CO Assay Buffer. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for two months at -20°C.
- **$H_2O_2$ Standard:** Ready to use. Stable for two months at -20°C.
- **Choline Oxidase:** Reconstitute the vial in 200 µl of PBS with 10 mM EDTA. Divide the reconstituted choline oxidase solution into aliquots and store at 4°C.

## VII. Choline Oxidase Activity Assay Protocol:

**1. Sample Preparation:** Grow bacteria in 500 ml of any suitable growth media (i.e. LB or any other media) overnight at 37°C. After incubation, harvest the cells by centrifuging at 10,000 x *g* for 20 min. Add 5 ml ice-cold PBS per 1 gram of wet cell pellet. Sonicate the cells for 5 min on ice and centrifuge at 10,000 x *g* for 20 min at 4°C. Transfer the clear supernatant to a new eppendorf tube. For each sample type, add 2-10 µl of the supernatant into two wells of a white, flat bottom 96-well plate labeled as "**Sample**" and "**Sample Background Control**". Adjust the volume to 50 µl/well using CO Assay Buffer. For **Positive Control** well, add 2 µl of reconstituted choline oxidase solution into the designated well and adjust the volume to 50 µl/well using CO Assay Buffer.

**Note:**

a) For Unknown Samples, we recommend doing a pilot experiment and testing several doses to ensure that the readings are within the linear range of the $H_2O_2$ Standard Curve.

**2. $H_2O_2$ Standard Curve Preparation:** Dilute the stock $H_2O_2$ Standard to 10 mM $H_2O_2$ Standard by adding 10 µl stock $H_2O_2$ Standard into 870 µl $dH_2O$. Dilute 10 mM $H_2O_2$ Standard to 0.1 mM $H_2O_2$ Standard by adding 10 µl of 10 mM $H_2O_2$ Standard into 990 µl $dH_2O$. Further, dilute the 0.1 mM $H_2O_2$ Standard to 10 µM $H_2O_2$ Standard by adding 100 µl of the 0.1 mM $H_2O_2$ Standard into 900 µl $dH_2O$. Add 0, 2, 4, 6, 8 and 10 µl of 10 µM $H_2O_2$ Standard into the desired wells of a 96-well, white plate to generate 0, 20, 40, 60, 80, 100 pmole $H_2O_2$ Standard/well. Adjust the volume of all Standard wells to 50 µl/well with CO Assay Buffer.

**3. Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix and 50 µl of Background Control Mix as indicated below:

| | Reaction Mix | *Background Control Mix |
|---|---|---|
| CO Assay Buffer | 39.5 µl | 49.5 µl |
| Choline | 10 µl | -- |
| CO Probe | 0.25 µl | 0.25 µl |
| CO Developer | 0.25 µl | 0.25 µl |

Mix well. Add 50 µl of **Reaction Mix** to wells containing **Standards**, **Sample(s)**, and **Positive Control**. Add 50 µl of ***Background Control** Mix to the **Sample Background Control well(s)**.

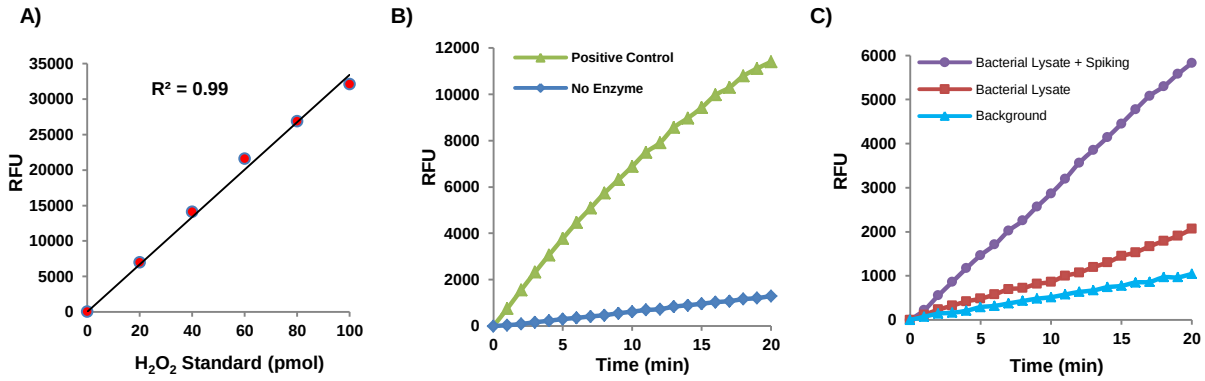
**4. Measurement:** Measure the fluorescence at Ex/Em= 535/587 nm in kinetic mode at 25°C for 20 min.

**5. Calculation:** Subtract 0 Standard reading from all Standard readings and Sample Background Control reading from all Sample readings respectively. Plot the $H_2O_2$ Standard Curve. Choose two time points ($t_1$ and $t_2$) in the linear portion of the curve for each Sample. Apply the corrected Sample readings to the $H_2O_2$ Standard Curve to get B nmol of $H_2O_2$ generated during the reaction time ($\Delta t = t_2 - t_1$). Calculate the Choline Oxidase activity of the Samples using the following equation:

**Sample Choline Oxidase activity = B x D / (Δt x V) = pmol/min/ml (mU/ml)**

Where: **B** is the amount of $H_2O_2$ from the Standard Curve (in pmol)
**Δt** is Reaction time (in min)
**V** is the sample volume added to the well (in ml)
**D** is the sample dilution factor (if applicable, D = 1 for Undiluted Samples)

**Unit Definition:** One unit is 1 µmole of $H_2O_2$ generated by choline oxidase per min at pH 7.5 and 25°C.




**Figures. A).** $H_2O_2$ Standard Curve. **B).** Reaction curve of Choline Oxidase activity (Positive Control vs Background). **C).** Reaction curve of Choline Oxidase in bacterial cell lysates before and after spiking with 0.2 mU of Choline Oxidase. Assay was performed according to assay protocol.

## VIII. Related Products:

Acetylcholinesterase Inhibitor Screening Kit (Colorimetric) (K197)
Choline Kinase Assay Kit (K476)
Butyrylcholinesterase Activity Kit (Colorimetric) (K516)
Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)
Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615)
Acetylcholinesterase Activity Colorimetric Assay Kit (K764)
Cholinesterase Activity Assay Kit (Colorimetric) (K975)

***FOR RESEARCH USE ONLY! Not to be used on humans.***