ab284569 – Choline Oxidase Activity Assay Kit (Fluorometric)

For determining the activity of choline oxidase in samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284569

Storage and Stability

On receipt entire assay kit should be stored at -20°C. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
Choline	1 vial	-20°C
Choline Oxidase	1 vial	-20°C
CO Assay Buffer	25 mL	-20°C
CO Developer	1 vial	-20°C
CO Probe	200 µL	-20°C
H2O2 Standard	100 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Briefly centrifuge all small vials at low speed prior to opening.

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 dH2O. 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.

H2O2 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

Assay Protocol

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.

Δ Note: *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 H2O2 Standard Curve.*

H2O2 Standard Curve Preparation:

- Dilute the stock H2O2 Standard to 10 mM H2O2 Standard by adding 10 µl stock H2O2 Standard into 870 µl dH2O.
- Dilute 10 mM H2O2 Standard to 0.1 mM H2O2 Standard by adding 10 µl of 10 mM H2O2 Standard into 990 µl dH2O.
- Further, dilute the 0.1 mM H2O2 Standard to 10 µM H2O2 Standard by adding 100 µl of the 0.1 mM H2O2 Standard into 900 µl dH2O.
- Add 0, 2, 4, 6, 8 and 10 µl of 10 µM H2O2 Standard into the desired wells of a 96-well, white plate to generate 0, 20, 40, 60, 80, 100 pmole H2O2 Standard/well.
- Adjust the volume of all Standard wells to 50 µL/well with CO Assay Buffer.

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed.
2. 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

3. 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).

Measurement

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

Calculation:

1. Obtain Δ Subtract 0 Standard reading from all Standard readings and Sample Background Control reading from all Sample readings respectively.
2. Plot the H2O2 Standard Curve. Choose two time points (t1 and t2) in the linear portion of the curve for each Sample.
3. Apply the corrected Sample readings to the H2O2 Standard Curve to get B nmol of H2O2 generated during the reaction time (Δt = t2-t1).
4. Calculate the Choline Oxidase activity of the Samples using the following equation:

   Sample Choline Oxidase activity = $B \times D / (\Delta t \times V)$ = pmol/min/mL (mU/mL)

   Where:

- B is the amount of H2O2 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 H2O2 generated by choline oxidase per min at pH 7.5 and 25°C.

Technical Support

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