Superoxide Dismutase

SOP derived from protocol developed from Counihan et al., 2019 and adapted by Ly Vuthy, UW- Tacoma.

Prepare solutions prior to assay.

SOD Homogenization Buffer:

15ml HEPES
7.5ml EGTA
28.69g mannitol
17.97g sucrose
750ml deionized water
Autoclave and store at 4°C

100mM EGTA:

3.80g EGTA
100ml deionized water
Add NaOH to increase pH until salt is in solution
Autoclave and store at 4°

Homogenize half the digestive gland in SOD homogenization buffer at a 1:5 weight:volume ratio

1. Centrifuge the homogenate at 1,500 x g at 4°C for 5 minutes
2. Collect the supernatant. Run in assay immediately or store at -80°C and run within 1 month of extraction.
3. Follow the instructions for the Cayman SOD kit (#706002) to assay sample extracts

Note: the following preparation is for a 96 well plate. It is not recommended to batch- make anything but the buffers as each reagent has an optimal window of effectiveness that differs from another. This assay requires samples and standards be assayed in duplicate and the presence of a sample background. Each well plate can support up to 40 samples.

4. Prepare Assay Buffer and Sample Buffer. These preparations are enough for 96 wells.
    a. Assay Buffer. Dilute 3ml Assay Buffer concentrate with 27ml HPLC-grade water. Store at 4C for up to two months.
    b. Sample Buffer. Dilute 2ml of Sample Buffer concentrate with 18ml of HPLC-grade water. Store at 4C for up to six months.
5. Prepare Standards.
    a. Dilute 20ul of the SOD Standard with 1.98ml of the diluted Sample Buffer to obtain SOD Stock solution.
    b. Take seven glass tubes and label them A-G. Follow the instructions in the table below to obtain the appropriate standards.

Tube	SOD Stock (ul)	Sample Buffer (ul)	Final SOD Activity (U/ml) in Well
A	0	1000	0

B	20	980	0.005
C	40	960	0.010
D	80	920	0.020
E	120	880	0.030
F	160	840	0.040
G	200	800	0.050

6. Bring Radical Detector, Standards and buffers to room temperature.
7. Prepare Radical Detector. In a foil covered tube, dilute 50ul of Radical Detector in 19.95ml of the diluted Assay Buffer. This is stable for two hours. Store unused Radical Detector at -20C.
    a. Put Xanthine Oxidase on ice. Ready one falcon tube with 1.95ml of diluted Sample Buffer. Put this on ice. When ready, add 50ul of Xanthine Oxidase to the falcon tube and mix by gently swirling. Do not vortex. This is stable for up to one hour and must be kept on ice until ready to use. The thawed Xanthine Oxidase cannot be refrozen. Unused reagent must be discarded.
        i. It is suggested that you make this reagent just before you load your plate with sample and buffer as outlined below.
8. Load the Plate
    a. Total well volume is 230ul.
    b. Add 200ul of the diluted Radical Detector to all wells, including the blank.
    c. All wells load 10ul of standard/ sample. If diluting the sample, use Sample Buffer and the sample volume is always 10ul.
    d. Sample background should have 30ul of Sample Buffer added and NO Xanthine Oxidase.
    e. Make Xanthine Oxidase reagent as outlined in step 8.
    f. Incite the reactions by adding 20ul of Xanthine Oxidase to all wells except for the sample background. This must be done quickly. Note the time you start adding the Xanthine Oxidase.
    g. Carefully shake the plate to mix and add the plate cover.
    h. Incubate at room temperature on a plate shaker for 30 minutes.
    i. Read the absorbance at 440-460nm. Note all readings done at 25C and 450nm.