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### **Trolox Equivalent Antioxidant Capacity Assay**

1. Prepare 1.5 mM Trolox stock solution: dissolve 7.5 mg Trolox in 20 ml PBS (pH 7.4). Gentle sonification is required to dissolve the crystals. After the crystals are completely dissolved, aliquot 1 ml solution to 1.5 ml Eppendorf microcentrifuge tubes and store at -80°C. The frozen stock solution is stable for more than 6 months.
2. Prepare 7mM ABTS radical cation stock solution:
  - a. Dissolve 8 mg ABTS in 1 ml water (solution A).
  - b. Dissolve 13.2 mg potassium persulfate in 10 ml water (solution B).
  - c. Mix 0.5 ml A and 0.5ml B above, and allow to stand in dark at room temperature for 12-16 hours before use. The concentrations of ABTS and potassium persulfate are in the mixture and 7 mM and 2.45 mM, respectively. The ABTS radical cation in this form is stable for at least two days.
3. Prepare fresh ABTS radical cation working solution: dilute 7 mM ABTS radical cation stock solution with PBS (pH 7.4) to an absorbance of 0.40 at 734 nm on the plate reader.
4. Preparation Trolox standard curve: using the following chart to prepare Trolox standard curve using 1.5 mM Trolox stock solution and PBS.

Tube	1.5 mM Trolox stock solution (μl)	PBS (μl)	Final Concentration (mM)
A	0	1000	0.000
B	30	970	0.045
C	60	940	0.090
D	90	910	0.135
E	120	880	0.180
F	150	850	0.225
G	180	820	0.270
H	220	780	0.330

5. Dilute samples with PBS, pH 7.4
6. Add 10 μl sample or Trolox standard to each well of the microplate
7. Add 200 μl ABTS radical cation work solution to each well the microplate

8. Shake and incubate the plate at 25 °C for 5 min.
9. Read the absorbance at 734 nm on plate reader.