Oxidative Enzyme Analysis using L-DOPA as the substrate

**Reagents**

**Acetate buffer (Stock Solution; 1 M)**

*In a 500 ml flask*

Fill two-thirds with DI water
68.04 g Sodium acetate buffer
Using a stir bar, bring to pH 5 (or the pH of your sample) by adding concentrated HCl
Transfer to 500 ml volumetric flask and bring to volume with DI water
Store at 4°C and use within 7 days – this is enough for 75 samples

**Acetate buffer (Working Solution; 50 mM)**

*In a 2 liter volumetric flask*

100 ml Acetate buffer stock solution
Bring to volume with DI water
Two liters is enough for 15 samples. Put in the 20 liter carboy for easy dispensing.

**Hydrogen Peroxide**

*In 100 ml volumetric flask*

10 ml H₂O₂ (3%)
Bring to volume with DI water
Make fresh each day

**Substrates**

*In a 100 ml opaque bottle*

Warm (90°C) 50 ml of DI water in a microwave (~30 sec)
246.5 mg L-DOPA (25 mM)

Mix well and allow to reach room temperature before analysis
Because L-DOPA is light sensitive, store in opaque container and mix the day of analysis

**Pre-Preparation**

Each well plate will hold three samples, but assays only one enzyme. Label Phenol Oxidase and Peroxidase ID code is PO and PE, respectively and use clear well plates.

**Procedure**

**Slurry**

*In 125ml wide mouth bottle*

1 g Soil or 0.5g Litter
Store at 4°C

Because enzymes activities have a negative relationship with time in buffer solution, make sure each sample is in the buffer for approximately the same amount of time (e.g. ±10min). For example, mix the samples prior to analysis and not all of them at once.
Add 125 ml of Acetate buffer (50mM)
Using a tissue homogenizer & timer, mix samples for 1 min
Pore slurry into dish and place on a stirrer (low speed)

**Enzyme Assays**
See L-DOPA oxidative enzyme map for illustration

Make sure to add the L-DOPA last for phenol Oxidase, or L-DOPA & H₂O₂ for Peroxidase.

Oxidative enzymes incubation can be from 4 h to 24 h depending on sample. Read when sample starts looking dark, but not too dark (abs >3.5)

Measure absorbance @ 460 nm

**Calculations**
From DeForest (2009) Soil Biology and Biochemistry

Enzyme activities were expressed in units of μmol h⁻¹ g⁻¹ and calculated by the follow equation:

\[
\text{Activity (μmol h}^{-1} \text{ g}^{-1}) = \frac{\text{Final ABS} \times 125 \text{ ml}}{(7.9 \mu \text{mol}^{-1}) \times 0.2 \text{ ml} \times \text{Time (hr)} \times \text{Soil (g)}}
\]

Where:

Final ABS = ABS of sample assay well s - ABS of negative control wells - ABS of sample control

ABS is the absorbance at 450 nm, 125 ml refers to the volume of the soil slurry, 7.9 μmol⁻¹ is the μmolar extinction coefficient, and 0.2 ml is the sample volume in the microplate. Soil is the oven-dried weight.

Note: Determining peroxidase and phenol oxidase with L-DOPA as the substrate have significant limitations. Refer to Johnsen & Jacobsen, SBB (2008) for details.