**Acetylcholinesterase**

**PROCEDURES**

1. **Sample preparation.** Blood samples should be diluted 40-fold in the Assay Buffer, e.g. accurately pipet 5 µL blood and mix thoroughly with 195 µL Assay Buffer. Tissue or cell lysates are prepared by brief sonication or homogenization in 0.1M phosphate buffer (pH 7.5), followed by centrifugation at 14,000 rpm for 5 min. Use supernatant for assay. Ideally samples should be assayed fresh. If this is not possible, refrigerate samples and assay them within 24 hours.

2. **Reagent preparation:** the Working Reagent should be prepared freshly and used within 30 min. Each reaction well requires 2 mg reagent. Calculate the amount of reagent needed and weigh this amount (mg) in a centrifuge tube. Add 200 µL Assay Buffer per 2 mg reagent. Vortex to dissolve.

   1. **Calibrator:** transfer 200 µL water and 200 µL calibrator separately into wells of a clear bottom 96-well plate.
   2. **Samples:** add 10 µL sample per well in separate wells.

3. **Reaction:** transfer 190 µL freshly prepared Working Reagent to all sample wells and tap plate briefly to mix.

4. **Read OD** at 2 min and at 10 min in a plate reader.

**Calculation:**

**AChE Activity =** \( \frac{OD_{10} - OD_{12}}{OD_{CAL} - OD_{H2O}} \times 200 \) (U/L)

Where \( OD_{n} \) and \( OD_{d} \) are the \( OD_{412nm} \) values of the sample at 10 min and 2 min, respectively. \( OD_{CAL} \) and \( OD_{H2O} \) are the \( OD_{412nm} \) values of the Calibrator and water at 10 min. \( n \) is the dilution factor (\( n = 40 \) for whole blood). The number "200" is the equivalent activity of the calibrator under the assay conditions.

**Note:** if the AChE activity without consideration of the dilution factor is higher than 600 U/L, dilute sample further in Assay Buffer and repeat the assay. Multiply the results by the dilution factor.

**Unit definition:** one unit of enzyme catalyzes the production of 1 µmole of thiocholine per minute under the assay conditions (pH 7.5 and room temperature).

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

**GENERAL CONSIDERATIONS**

1. **This assay is based on an enzyme-catalyzed kinetic reaction.** Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

2. **For assays in standard 1 mL cuvet, use 1 mL water and 1 mL Calibrator, 50 µL sample + 950 µL Working Reagent.**

**EXAMPLES**

Two human blood samples were assayed in duplicate using the 96-well plate protocol. The AChE activities were 3,402 ± 163 and 3,660 ± 151 U/L.

**PUBLICATIONS**

