1. Addition of Assay Buffer
Add 50 μL of the Assay Buffer (ORANGE Dot) to each assay well.

2. Addition of Controls or Samples
Add 25 μL of the appropriate control or sample to each assay well.
Swirl wells to mix for 15 seconds.

3. Addition of Diluted Oxidizer
Add 25 μL of diluted oxidizer to each assay well.
Swirl wells to mix for 15 seconds.
Incubate for 5 minutes at 70 degrees F +/− 20 degrees.

4. Addition of Neutralizer
Add 25 μL of neutralizer (RED dot) to each assay well.
Swirl wells to mix for 15 seconds.

5. Addition of Enzyme
Add 25 μL of ACh-E (GREEN dot) to each assay well.
Swirl wells to mix for 15 seconds.
Incubate for 15 minutes at 70 degrees F +/− 20 degrees.

6. Addition of Substrate
Add 25 μL of Substrate-ATC (BLUE dot) to each assay well.
Swirl wells to mix for 15 seconds.

7. Addition of Chromogen
Add 25 μL of Chromogen-DTNB (YELLOW dot) to each assay well.
Swirl wells to mix for 15 seconds.
Incubate for 30 minutes at 70 degrees F +/− 20 degrees.

8. Addition of Stop Solution
Add 25 μL of Stopping Solution (PURPLE dot) to each assay well.
Swirl wells to mix for 15 seconds.

9. Interpret Results
Read at 405nm (optimum wavelength) or 450nm. Be sure no bubbles are visible in any well as they will cause erroneous readings.

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911  Fax: 215-357-5232  www.abraxiskits.com
1. **ACh-E**
   Transfer 3ml from the 7ml Petri vial (GREEN dot) containing ACh-E Diluent and place into the 7ml Petri vial (GREEN dot) containing lyophilized ACh-E.
   Allow at least 5 minutes for the ACh-E to go into solution before using in the Assay.

2. **Substrate (ATC)**
   Transfer 3ml from 7ml Petri vial (BLUE dot) containing ATC Diluent and place into 7ml Petri vial (BLUE dot) containing lyophilized ATC.
   Allow at least 5 minutes for the ACh-E to go into solution before using in the Assay.

4. **Addition of Oxidizer**
   Determine the amount of Diluted Oxidizer needed for the Assay.
   Dilute the Oxidizer (AMBER bottle with ORANGE dot) 1 part Oxidizer to 9 parts Assay Buffer / Oxidizer Diluent (ORANGE dot) and mix by shaking moderately.
   This diluted oxidizer must be made fresh for each Assay.

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