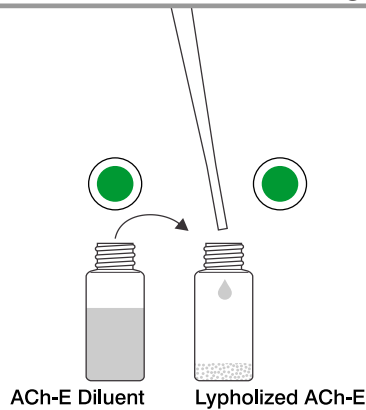


1. ACh-E

Transfer 3ml from the 7ml Petri vial (GREEN dot) containing ACh-E Diluent and place into the 7ml Petri vial (GREEN dot) containing lypholized ACh-E.

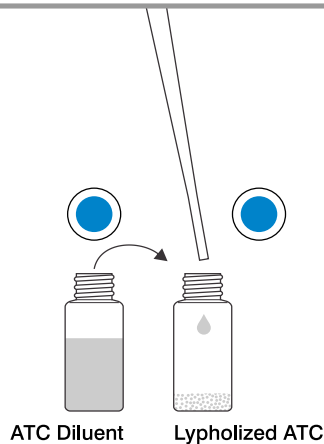
Allow at least 5 minutes for the ACh-E to go into solution before using in the Assay.



2. Substrate (ATC)

Transfer 3ml from 7ml Petri vial (BLUE dot) containing ATC Diluent and place into 7ml Petri vial (BLUE dot) containing lypholized ATC.

Allow at least 5 minutes for the ACh-E to go into solution before using in the Assay.

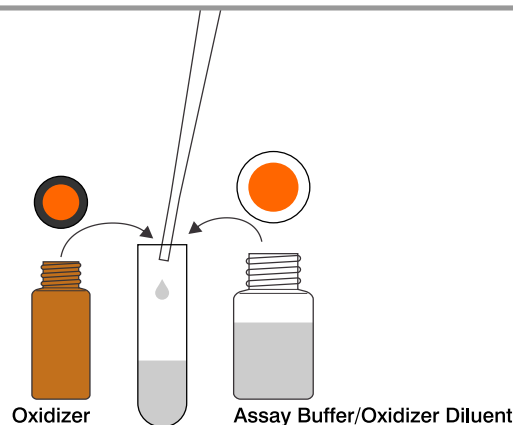


4. Addition of Oxidizer

Determine the amount of Diluted Oxidizer needed for the Assay.

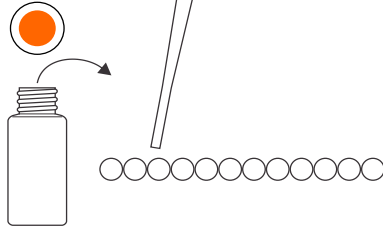
Dilute the Oxidizer (AMBER bottle with ORANGE dot) 1 part Oxidizer to 9 parts Assay Buffer / Oxidizer Diluent (ORANGE dot) and mix by shaking moderately.

This diluted oxidizer must be made fresh for each Assay.



1. Addition of Assay Buffer

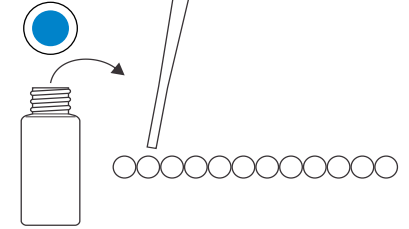
Add 50 uL of the Assay Buffer (ORANGE Dot) to each assay well.



6. Addition of Substrate

Add 25 uL of Substrate-ATC (BLUE dot) to each assay well.

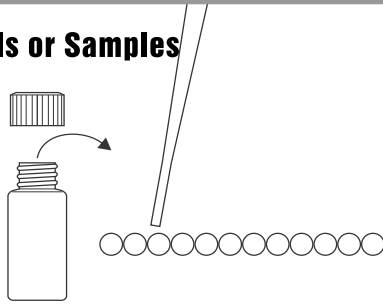
Swirl wells to mix for 15 seconds.



2. Addition of Controls or Samples

Add 25 uL of the appropriate control or sample to each assay well.

Swirl wells to mix for 15 seconds.

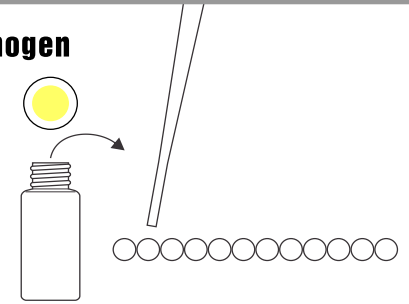


7. Addition of Chromogen

Add 25 uL 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.

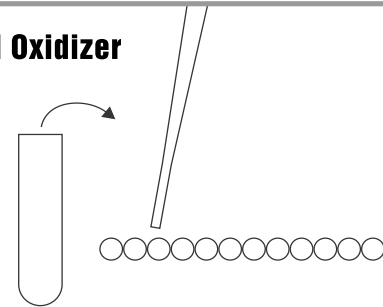


3. Addition of Diluted Oxidizer

Add 25 uL of diluted oxidizer to each assay well.

Swirl wells to mix for 15 seconds.

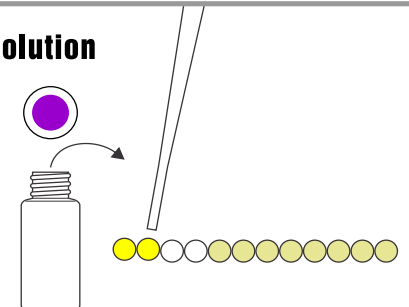
Incubate for 5 minutes at 70 degrees F +/- 20 degrees.



8. Addition of Stop Solution

Add 25 uL of Stopping Solution (PURPLE dot) to each assay well.

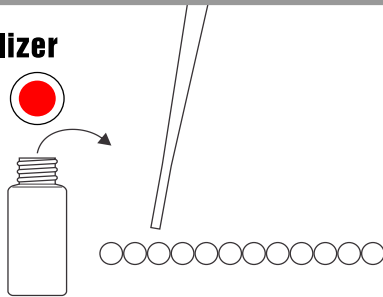
Swirl wells to mix for 15 seconds.



4. Addition of Neutralizer

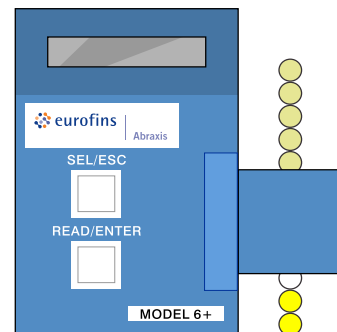
Add 25 uL of neutralizer (RED dot) to each assay well.

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.



5. Addition of Enzyme

Add 25 uL 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.

