Acetic Acid Test Kits (07/2011)

Q1. What are the major the differences between the various acetic acid test kits?

Megazyme produces 4 acetic acid test kits:

EK-ACET: uses the traditional ACS reaction. Manual format for use with spectrophotometers.

EK-ACETAF: uses the traditional ACS reaction. Automated format for use with auto-analysers.

EK-ACETAK: uses the more recently developed and more rapid acetate kinase reaction. Automated format for use with auto-analysers.

EK-ACETRM: uses the more recently developed and more rapid acetate kinase reaction. Manual format for use with spectrophotometers.

Q2. Which acetic acid kit is recommended for a 96-well microplate format?

Auto-analysers use ~ 0.315 mL reaction volumes and pathlengths between 4-8 mm which is similar to a standard 96-well microplate where a 0.315 mL reaction volume would give a pathlength of ~ 6-7 mm. Therefore **EK-ACETAK** or E**K-ACETAF** can be used directly in a 96-well microplate format with minimal assay optimisation.

If preferred, **EK-ACET** or E**K-ACETRM** may also be easily converted for use in a 96-well microplate format. Basically, the assay volumes for the cuvette format must be reduced approximately 10-fold for use in a 96-well microplate. However, some assay optimisation may be required (e.g. increased enzyme concentration etc.) and unlike the cuvette which has a set pathlength of 1 cm, the pathlength in the microplate is dependent upon the volume of liquid in the well. Therefore to enable the calculation of the amount of analyte in the samples from tests performed in the microplate format one of the following must be done:

1. The easiest method is to use a microplate reader that has a pathlength conversion capacbility (i.e. the microplater reader can detect the pathlength of each well and convert the individual readings to a 1 cm pathlength). This will allow values to be calculated using the MegaCalc calculation software which can be found where the product is located on the Megazyme website.

2. Perform a standard curve of the analyte on each microplate that contains test samples and calculate the result of the test samples from the calibration curve (concentration of analyte versus absorbance).

3. Perform a standard curve of the analyte in both the cuvette format (i.e. with a 1 cm pathlength) and the 96-well microplate format and use these results to obtain a mean conversion factor between the cuvette values and the microplate values.

Acetic Acid Kit Recommendation For Microplate Format:

Either E**K-ACETRM** or E**K-ACETAK** is recommended for use in a 96-well microplate format and ~~the main adv~~antages / disadvantages are described below:

EK-ACETRM:
The assay volumes of this kit should be reduced by 10-fold for use in a 96-well microplate format (some assay optimisation may be required e.g. increased enzyme concentration etc.).

The calculation of results is achieved as outlined above in either of points 1, 2 or 3.

The main advantage here is that if this kit is used with a microplate reader that has a pathlength conversion capability or if results are converted as outlined above in point 3 then this enables easy calculation of results using the **EK-ACETRM** MegaCalc application (available on the Megazyme website where the product is located).

~~EK-ACETAK~~:
This kit is designed for use in an auto-analyser and therefore **can be used without any modification to assay volumes** directly in a 96-well microplate format.

This kit has less reagent additions than E**K-ACETRM**.

EK-ACETAK does not have a MegaCalc application available to enable easy results calculation which therefore must be achieved as outlined above in either of points 2 or 3.

Q3. Does the decolourizing preparation remove some VA during the process?

No, however the sample preparation process can be tested by using adding a known amount of acetic acid standard and assessing the recovery of this.

Q4. Can acetic acid be measured in culture / fermentation media?

Acetic acid in liquid cell culture media / supernatants or fermentation samples can be determined without any sample treatment (except clarification by centrifugation or filtration and appropriate dilution in distilled water.