Ethanol, UV method

Alternative Procedures

Introduction

This kit has been developed to work in cuvettes with a standard pathlength of 1 cm, as described in the respective "Product Brochure". However, it can be adapted for use in 96-well microplates or in auto-analysers (micro-volume formats) with minimal assay optimisation. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than the standard cuvette pathlength of 1 cm. Thus, to perform the calculation of the amount of analyte in the samples under analysis follow one of the three strategies described in the section below.

Strategies for analyte calculation

Auto-analysers use reaction volumes of approximately 0.315 ml and pathlengths from 4 to 8 mm, which is similar to a standard 96-well microplate in which the same reaction volume would have a pathlength of 6 or 7 mm (similar assay volumes). Therefore, in both formats (96-well microplate and auto-analysers systems), the calculation of the analyte must be done by one of the three possible methods described below:

1. Using the pathlength conversion factor

This is the easiest method to perform the calculation of the analyte. However, it requires a microplate reader with pathlength conversion capacity, i.e., the apparatus can detect the pathlength of each well and convert the individual readings to a 1 cm pathlength (cuvette format). In the case of auto-analysers, the absorbance readings should be directly converted to a 1 cm pathlength. This will allow the calculation of the analyte content as described in the "Product Brochure", provided with the kit and available at the NZYTech website.

2. Using one standard curve

In this method, it is necessary to perform a standard curve of the analyte on each microplate that contains the test samples, or in the auto-analyser, and calculate the result from the standard curve of analyte concentration vs. absorbance. The standard curve can be performed by using the control solution provided in the kit.

3. Using two standard curves

The most complicated method is to perform standard curves of the analyte in both the cuvette format (i.e. with a 1 cm of radiation pathlength) and the 96-well microplate or auto-analyser formats, and use these results to obtain a mean conversion factor between the cuvette procedure values and the alternative procedure values.

The standard curves can be performed by using the control solution provided in the kit.

Procedure (microplate)

Wavelength: 340 nm

Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)

Temperature: ~ 25ºC

Final volume: 0.254 ml

Sample solution: 0.025-1.2 µg of ethanol per cuvette (in 0.010-0.200 ml sample volume)

Pipette into cuvettes (mL)	**Blank**	**Sample**	**Standard**
Distilled water	0.210	0.200	0.200
Sample	-	0.010	-
Standard solution	-	-	0.010
Solution 1 (PP_i buffer)	0.020	0.020	0.020
Solution 2 (NAD^+)	0.020	0.020	0.020
Suspension 3 (Al-DH)	0.002	0.002	0.002
Mix, measure the absorbance of the solutions (A1) after ~2 min and start the reaction by addition of			
Suspension 4 (ADH)	0.002	0.002	0.002
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 5 min)*			

Mixture can be obtained using microplate shaker, shake function on a microplate reader, or repeated aspiration (e.g. using a pipettor set at 50 - 100 µL volume).

* if necessary, continue to read the absorbances at 1 min intervals until the reaction ends.

Calculation (microplate procedure)

$$g/L = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times g/L\ standard \times F$$

If the sample is diluted during preparation, the result must be multiplied by the dilution factor, F.

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