THE DETERMINATION OF 4-METHYL UMBELLIFERONE USING THE MODEL LS-50 WELL PLATE READER ACCESSORY

Many modern immunological assays involve the enzyme-catalyzed hydrolysis of non-fluorescent 4-methylumbelliferone phosphate to the highly fluorescent 4-methylumbelliferone (4mU) product. In this application the enzyme acts as a biological amplifier, producing many molecules of fluorescent product for each original analyte molecule.

4 mU has been analyzed using the PerkinElmer Model LS-50 Luminescence Spectrometer fitted with the Well Plate Reader accessory (L225-0137) to evaluate linearity and sensitivity characteristics.

METHOD

4-Methylumbelliferone free acid (M-1381) and glycine buffer, 50 mM pH 10.4 (105-2) were obtained from Sigma Chemical Co.

A calibration series of 4 mU samples in glycine buffer was prepared to give a concentration range of 1 to 1250 picomoles per well.

INSTRUMENT CONDITIONS

Model LS-50        Ex 350 nm Em 465 nm
                   Slits 10/10 nm
                   Scan speed 480 nm/min

RESULTS

The excitation and emission spectra of 4 mU in glycine buffer are shown in Figure 1. The samples were placed in a 96-well microplate. Excitation and emission maxima obtained from Figure 1 were used to prepare the calibration graph shown in Figure 2. The assay was found to be linear over the concentration range 1 to 1250 picomoles per well using a sample volume of 250 µL. The calibration graph was prepared using Enzfitter*: Plate Reader data is also fully compatible with ELISAsoft, a PC based microplate reader analysis package.

The limit of detection, defined as that concentration of analyte giving a signal-to-noise twice that of the background, was calculated as approximately 1 picomole per well. This detection limit is comparable to that obtained using dedicated twin monochromator plate readers.

*Enzfitter is a product of Elsevier-Biosoft