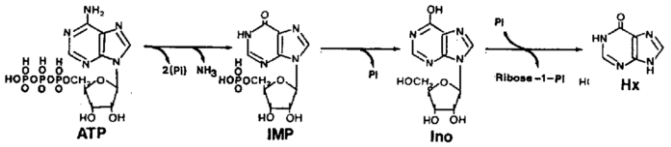
Microplate Spectrophotometric Enzymatic
PRECICE® Nucleotides Assay Kit

Item # : K0700-003-22



Kit Specifications:

Format:	96-well plate
Tests per kit:	22 samples
Type:	Quantitative
Method:	Enzymatic reactions
Incubation time:	30 minutes

Principle:

PRECICE® Nucleotides Assay Kit has been specifically designed to facilitate the measurement of ATP degradation in fish muscle samples. This method employs specific dehydrogenases that convert IMP (enzyme 1), hypoxanthine (enzyme 2) and inosine (enzyme 3) to NADH. After incubation with the enzymes, NADH is quantitatively measured at a wavelength of 340nm. The absorbance at 340nm developed in the presence of Enzyme 1, Enzyme 2 or Enzyme 3 is directly proportional to the amount of IMP, of hypoxanthine and of inosine respectively. These absorbance data can be used to calculate K-value, H-value or other values:

$$K_i(\%) = \left[\frac{\text{Ino} + \text{Hx}}{\text{IMP} + \text{Ino} + \text{Hx}}\right] \times 100$$

$$H(\%) = \left[\frac{\text{Hx}}{\text{IMP} + \text{Ino} + \text{Hx}}\right] \times 100$$

$$F_r(\%) = \left[\frac{\text{IMP}}{\text{IMP} + \text{Ino} + \text{Hx}}\right] \times 100$$

References:

Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E., Toyama, T. Determination of fish freshness with an enzyme sensor system. 1984 ***J. Agric. Food Chem.*** 32, pp.314-319

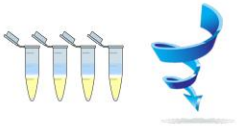
Gill, T.A. Thompson, J.W., Gould, S.& Sherwood, D. 1987 Charcaterisation of quality deterioration of yellow fin tuna. ***J.Food Sci. 52***, pp. 580-583

Luong, J.H.T., Male, K.B., Masson, C., & Nguyen, A.L. 1992. Hypoxanthine ratio determination in fish extract using capillary electrophoresis and immobilized enzymes. ***J. Food Sci.***, 57, pp. 77 - 81.

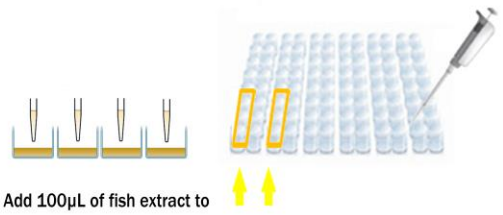
Overview:

1. Weight 5-20g of fish muscle Add 1V per gram of Extraction buffer

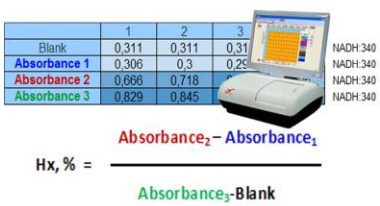
2. Boil for 20min, centrifuge 5 min



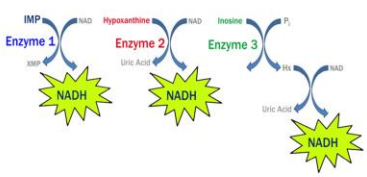
3. Dilute 7-fold* in Extraction buffer, centrifuge 5 min



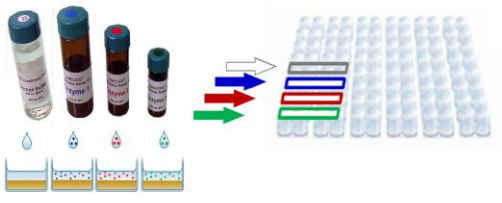
4. Add 100µL of fish extract to four separate wells



7. Shake and read Optical Density at 340nm, interprete data



6. Shake and incubate for 30 min



5. Add 100µL of Blank, Enzyme 1, Enzyme 2 and Enzyme 3 per well