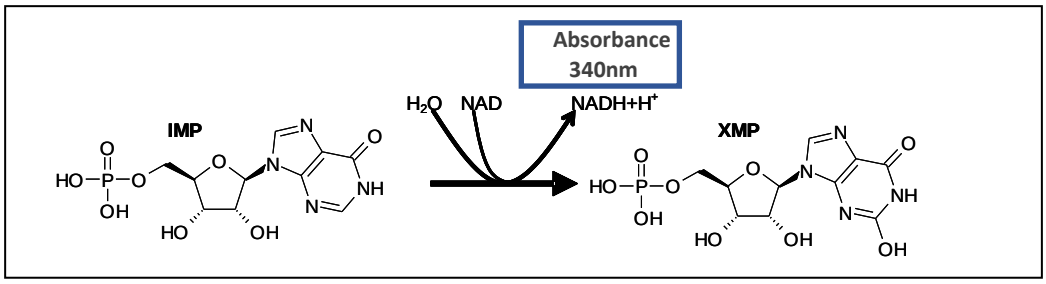
PRECICE® IMP Assay Kit

for enzymatic determination of IMP nucleotide in fishmeal samples - Ref. 0700-006

I. Principle

PRECICE® IMP Assay Kit provides an enzymatic tool for measuring IMP content samples by long-UV spectrophotometry.



PRECICE® IMP Assay Kit is based on the use of recombinant IMP-specific dehydrogenase that allows irreversible and quantitative conversion of **IMP** to **NADH**. The NADH formed can be quantified by measuring specific absorbance at 340nm. For maximal accuracy, the assays are run with and without IMPDH enzyme in parallel. The absorbance rate observed in the absence of IMPDH is used as blank and is subtracted from the absorbance rate measured in its presence.

II. Materials and equipment required (not provided)

1) Autoclaved deionized water;
2) 5-10ml self-standing vials with caps for fishmeal powder solution;
3) 1,5mL centrifuge tubes;
4) 13 000 RPM centrifuge (type Biofuge)
5) Plate reader fitted with a filter 340nm (ex. Labsystems iEMS Reader MF (Thermo), Epoch (BioTek); PerkinElmer);
6) Slow-rotating plateform.

III. Kit Content for 12 samples:

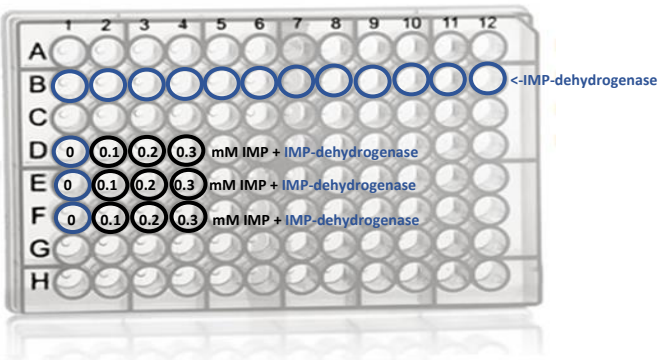
Once dissolved, the reagents provided in the kit are not stable and should be used the day of preparation.

A standard **PRECICE® IMP Assay Kit** contains contains:

- one sealed 96-well microplate (round-bottom 96-well plate Corning, Costar®, ref. 3797) prefilled with **IMP-dehydrogenase enzyme**

and **IMP standards (3 lanes);**

- one tube **"Cofactor 1";**
- one tube **"Cofactor 2";**
- one vial **"Reaction buffer"**;



IV. Storage

PRECICE® IMP Assay Kit is shipped at room temperature provided in stable lyophilized form allowing the transport at room temperature. Once received, the kit must be stored at -20°C until used.

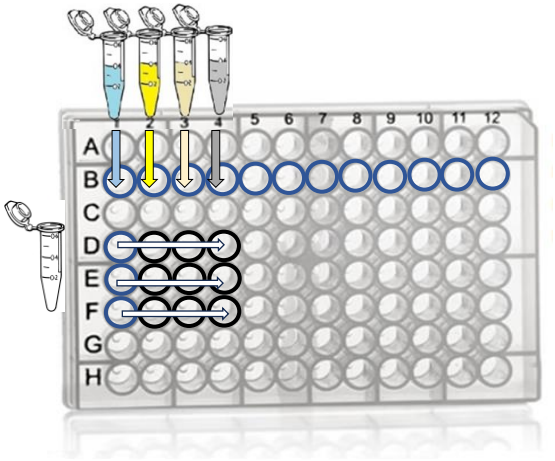
V. Protocol:

For dry fishmeal samples:

1. Weight 150-200 mg of fishmeal powder in 5ml vials;
2. Calculate the volume of water to produce 40mg/ml concentration;

For liquid samples:

3. Weight 150-200 mg of liquid viscous sample (3-4 drops) in 10-15ml vials;
4. Calculate the volume of water to produce 20mg/ml fishmeal concentration;
5. Add calculated volume of deionized autoclaved water (control the volume of added water by weight, using 1g=1mL);
6. Put on rotating platform for 1h at room temperature;
7. Pour ~1 ml of fishmeal suspension to a clean, labeled 1.5ml tube and clarify by centrifugation at 13 000 rpm, for 10 min.
8. To prepare **"Reaction buffer"**, quantitatively transfer cofactors (powder) to **"Reaction buffer"** tube. To do it, add 1mL of Reaction buffer to each tube, close and mix by inverting until complete powder solubilization, put it back to **"Reaction buffer"** tube. Close and invert several times until complete powder dissolution. Repeat.
9. Dispense 500µL of **"Reaction buffer"** with cofactors into 12 numbered clean tubes (for 12 samples or for 6 samples in duplicate). Add 0.5mL of fishmeal clarified extract.
10. To realize a calibration curves, add 500µL of **"Reaction buffer"** to 500µL of deionized water (for one calibration).
11. Dispense 200µL of each extract diluted in reaction buffer to 2 wells (Lane A and lane B of the same column (example: 200µL of Sample 1 to A1 and B1 wells, change the tip and dispense 200µL of Sample 2 etc).
12. Dispense 200µL of Reaction buffer diluted with water to 4 wells with IMP (calibration curve).
    You can do it in duplicate or triplicate.
13. Once microplate is filled, inspect for the absence of air bubbles that could disturb reading. Air bubbles can be removed by gentle blowing cold air with hair drier (few seconds);
14. Shake microplate for 1 min x at 2-3* times, insert it in microplate reader;



15. Program microplate reader at 340nm (Kinetics mode, every min, 30°C) and follow the reaction for 15 min at 340nm. During this time, optical density signals should achieve the plateau.

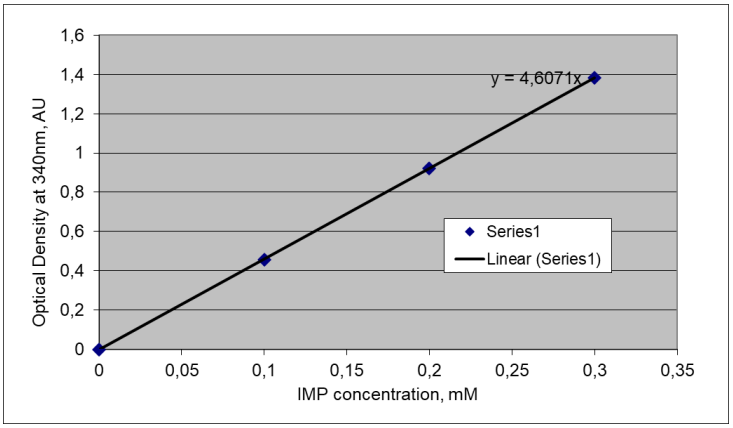
16. Once plateau is achieved, stop the reaction, agitate for 1 min again and read optical density at 340nm.
    IMPORTANT: Rigorous prolonged shaking is essential for IMP and enzymes solubilization. It helps also to avoid elevated products concentrations at the bottom of the well.

VI. Calibration with IMP solutions

1. Subtract Blank value (D1, no IMP) from the values obtained with 0.1; 0.2 and 0.3mM IMP;

   Example of data obtained with iEMS (Thermo) microplate reader (ε=4.6):

	D1, no IMP	D2, 0,1mM IMP	D3, 0,2mM IMP	D4, 0,3mM IMP
OD340nm	0,184	0,640	1,105	1,568
After D1 subtraction	0	0,456	0,921	1,384



2. Plot these values against IMP concentration and calculate linear regression coefficient (ε) (ε may vary from 3.6 to 5 depending on microplate reader and lamp).

VII. Calculating IMP concentration in fishmeal

Calculate the concentration of IMP in the samples as follows:

$$\text{IMP } (mg/kg) = \frac{(Abs_B - Abs_A)*348}{\varepsilon*0.02}$$

where:

Abs_B - the absorbance in the well of the lane B containing IMP-dehydrogenase enzyme;

Abs_A - the absorbance in the well without enzyme (Blank);

0.02g/L - fishmeal concentration after 2-fold dilution of 40mg/ml suspension

ε - extinction coefficient for 1mM IMP in 200µL of round-bottom microplate (Corning, Costar® ref. 3797)

Example of data with fishmeal samples obtained with iEMS (Thermo) microplate reader using ε=4.6:

	Fishmeal 1	Fishmeal 2	Fishmeal 3	Fishmeal 4	Fishmeal 5	Fishmeal 6	Fishmeal 7	Fishmeal 8	Fishmeal 9	Fishmeal 10
A, OD340nm	0,729	0,614	0,758	0,743	0,62	0,601	0,821	0,661	0,616	0,858
B, OD 340nm	1,502	0,968	1,589	1,157	0,67	0,907	1,275	1,149	0,646	1,416
IMP, concentration mg/kg using PRECICE IMP Assay kit	2918	1336	3137	1563	189	1155	1714	1842	113	2106
HPLC IMP, g/kg	2,87	1,33	3,07	1,67	0,21	1,25	1,92	1,77	0,07	2,34

Correlation with HPLC r^2=0.99, recovery 98%