Freshness Assay Kit "*PRECICE® K*"

***For:** determining fish freshness through K value measurement*
quantifying the products of ATP degradation (IMP, inosine, hypoxanthine)
measuring changes in "umami" and "umami"-related substances

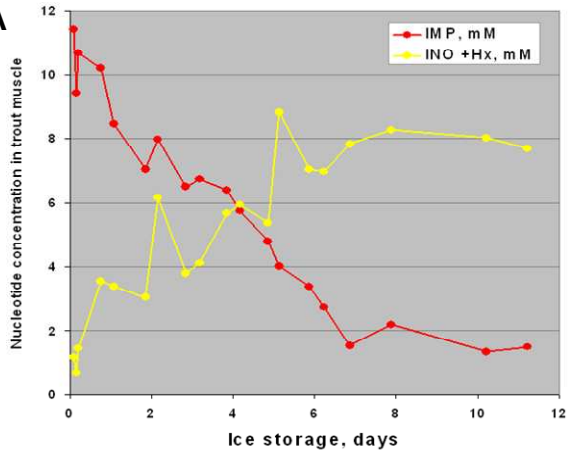
PRECICE® K-Freshness Assay Kit is the first enzymatic assay for a routine measurement of fish freshness in a convenient microplate format. Freshness is measured by determining the level of products formed from ATP degradation: IMP, inosine and hypoxanthine. In this assay, fish extracts are incubated for 60 min (in parallel with "blank" controls) with two enzymatic mixtures, allowing the complete conversion of IMP, inosine and hypoxanthine and the subsequent formation of NADH which is then easily measurable by absorbance at 340nm. Absorbance data are directly used for the calculation of K-value, a reliable and objective indicator of fish freshness. The assay allows the detection of degradation as low as 4% of IMP (equivalent to 10-12h of fish storage on ice).

- an ideal tool for a routine control of fish freshness,
- measurement of up to 32 fish samples at once in 60min using a standard microplate reader,
- easy to use ("add and measure"),
- very simple procedure for sample preparation, compatible with PCA extraction,
- high precision,
- fully validated for freshness measurement of tuna, salmon, trout and other major marketed species.

Kit Contents: A standard **PRECICE® K-Freshness Assay Kit** (one 96-well plate) contains:
- 15ml vial containing "Enzyme mix I" (orange), lyophilized, for IMP quantification
- 15ml vial containing "Enzyme mix II" (blue), lyophilized, for IMP + Ino + Hx quantification
- 50ml vial containing 20ml of "Reaction buffer"
- 3 vials containing cofactors (powder)
- a transparent microplate (round-bottom 96-well plate Corning, Costar®, ref. 3797)

Case study: Changes in content of ATP-breakdown products (IMP, inosine and hypoxanthine) of rainbow trout stored on ice as soon as slaughtered were characterized using **PRECICE® K-Freshness Assay Kit**. ~3g of fish muscle were collected and boiled for 20min. Fish extracts were recovered and diluted with 1 volume of water. 4µL of the diluted extract were put into 6 wells on a 96-well plate before adding 200µL of Reaction buffer, Enzyme mix I or Enzyme mix II, all in duplicate. After 1h of incubation at 37℃, abso rbance was measured at 340nm using an iEMS Reader MF (Labsystems) microplate reader. Absorbance data were used for the determination of nucleotide contents (Figure A) and for the calculation of K-value (Figure B). Orange bars in Figure B show the freshness measured by the same procedure for trout (T) and salmon (S1 and S2) samples provided by retailers.

A



B