Isopeptidase T from human erythrocytes

Product Number I1154
Storage Temperature –70 °C

EC 3.1.2.15
Synonyms: Ubiquitin Thiolesterase; Ubiquitin C-Terminal Hydrolase

Product Description
Isopeptidase T acts on polyubiquitin to release ubiquitin monomers. This enzyme, part of a family of deubiquinating cysteine hydrolases, is thought to play a major role in the recycling of free ubiquitin and in the regulation of the ubiquitin-proteasome pathway. It is a monomeric protein with a molecular weight of 100 kDa and is abundant in erythrocytes and reticulocytes.1,2 Iodoacetamide and ubiquitin aldehyde are inhibitors of isopeptidase T.1,2

Vial Content: 25 µg of enzyme in a frozen solution containing 50 mM HEPES buffer, pH 7.6, 1 mM DTT, 50 mM NaCl, 8 mM CHAPS, 0.1 mM EDTA, 10 µg/ml bestatin, and 2 µg/ml leupeptin.

Purity: ≥90% (SDS-PAGE)

Enzyme Activity: ≥20 units per mg protein (Bradford)

Unit Definition: One unit will release 1.0 nmole of 7-amino-4-methylcoumarin (AMC) from Z-Leu-Arg-Gly-Gly-AMC per minute at 25 °C at pH 7.6.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product is shipped on dry ice and should be stored at –70 °C. When stored at –70 °C this enzyme is stable for at least 2 years.

Procedure
The enzyme activity is determined by a fluorimetric assay. Isopeptidase T catalyzes the following reaction:

\[ \text{Z-Leu-Arg-Gly-Gly-AMC} \rightarrow \text{Z-Leu-Arg-Gly-Gly + AMC} \]

The assay measures the release of the fluorescent moiety (AMC) from the peptide substrate in the presence of 0.5 µM ubiquitin and 10 mM DTT.3

A small amount of ubiquitin is included in the reaction buffer, because submicromolar amounts have been shown to activate the enzyme. Higher concentrations are inhibitory.3

Solutions
Reaction Buffer: 28 mM HEPES, pH 7.6, containing 0.7 mM EDTA, 1.4 mg/ml ovalbumin, 14 mM DTT, and 0.71 µM (6 µg/ml) of ubiquitin (Product Number U6253)

Substrate Solution: 2.4 mM (1.7 mg/ml) Z-Leu-Arg-Gly-Gly-AMC, acetate salt in DMSO.

Inhibitor solution: 300 µg/ml ubiquitin aldehyde (Product Number U1507) in water (optional).

Fluorimetric Standard Solution: 10 mM (1.75 mg/ml) 7-amino-4-methylcoumarin (AMC, Product Number A9891) in DMSO. Dilute this solution 100-fold with the Reaction Buffer and measure the absorbance at 354 nm. Calculate the exact concentration of the solution using the extinction coefficient for AMC at 354 nm (E\(^{\text{mm}}\) = 16). Dilute an aliquot of this solution to 20 µM using the Reaction Buffer.
Enzymatic Assay Procedure
To a 96 well plate suitable for fluorescence, add to each well the following:

1. Prepare wells for the determination of a standard curve. To separate wells add 0, 10, 25, 50, 100, and 150 µl of the prepared 20 µM Fluorimetric Standard Solution and bring the total volume in each well to 200 µl with Reaction Buffer. The standard solution contains 20 nmoles per ml (20 µM) and the range of the standard curve will be 0.2 to 3.0 n mole of AMC per well.
2. Prepare wells for the enzyme assay. In the first well for the enzyme assay, place 40 µl of deionized water as a reagent blank.
3. Place in subsequent wells a suitable volume of the enzyme sample solution and bring the total volume to 40 µl with deionized water.
4. Add 140 µl of the Reaction Buffer and incubate at 37 °C for 15-20 minutes for activation of the enzyme by DTT and ubiquitin.
5. Start the reactions by adding 20 µl of the Substrate Solution to each well. Ensure no bubbles are present in the wells because they will adversely affect the results.
6. Place the plate in the fluorimeter plate reader.
   Fluorimeter settings:
   - Excitation wavelength: 380 nm
   - Emission wavelength: 440 nm
   - Slit width: 5 nm
7. Measure the Fluorescence Units (FLU). The reaction may be observed in real time, a kinetic program at 5 minutes intervals to check on the linearity of the reaction. (Recommended reaction time is 0-25 minutes)
8. Calculate the ΔFLU/minute from the linear portion of the reaction curve. For systems with other endogenous activities, the reaction rate (ΔFLU/minute) for Isopeptidase T is the value obtained in the sample without inhibitor minus the value obtained in the sample with inhibitor.
9. From the fluorescence measured from the wells containing the standard solutions, plot a standard curve of FLU against the amount of AMC present. From the Standard Curve determine the Fluorescence Units corresponding to 1 n mole of AMC (FLU/nmole).

Results

\[ \text{Units/ml} = \frac{(\Delta \text{FLU/minute}) \times (\text{dilution factor})}{(\text{nmole/min/ml})} \times \frac{\text{FLU/nmole} \times (V \ [\text{ml}])}{\Delta \text{FLU/minute}} \]

\(\Delta\)FLU/minute: value determined for enzyme assay in Step 8 minus the value determined for the blank assay.
Dilution Factor: any dilution of original protein sample prior to addition to well.
FLU/nmole: value determined from standard curve in Step 9
V = volume, in ml, of enzyme solution in reaction

References

NDH,JWM,MAM 11/05-1