Aurora Biolabs
High Purity Protein for Research

Enterokinase (EK) Activity Assay Kit

Catalog Number: 190006AK

Background

Enterokinase (EK), also known as enteropeptidase, is a highly specific serine protease. It possesses a distinct recognition sequence—Asp-Asp-Asp-Asp-Lys (DDDDK)—and catalyzes the hydrolysis of the peptide bond immediately following the lysine residue. This stringent substrate specificity makes EK an ideal molecular tool for bioprocessing and protein engineering, specifically for the site-specific removal of affinity tags from purified recombinant fusion proteins.

Assay Principle

The Enterokinase (EK) Activity Assay Kit is a fluorogenic assay designed to quantitatively measure EK protease activity. The kit includes a specific EK substrate (Asp-Asp-Asp-Asp-Lys) conjugated to the fluorophore AFC (7-amino-4-trifluoromethylcoumarin). Upon proteolytic cleavage of the substrate by EK, free AFC is released, generating a strong fluorescent signal. The fluorescence can be detected using a microplate fluorescence reader with excitation at 365 nm and emission at 505 nm. The increase in fluorescence intensity is directly proportional to EK enzymatic activity and can be used to calculate protease activity. Purified EK protease is provided in the kit as a positive control to validate assay performance and ensure proper assay setup.

Application

Measure Enterokinase (EK) protease activity.

Plate Reader

A microplate reader capable of measuring fluorescence intensity is required.

Components

Catalog number	Item	Amount	Storage
190006B	Assay buffer	25 mL	-20°C
190006	Purified EK protein	5 µL	-80°C
190006F	Fluorogenic substrate (1 mM)	100 µL	-80°C
190006S	5-AFC standard (1 mM)	10 µL	-80°C
	96-well microplate, black	1	Room temperature

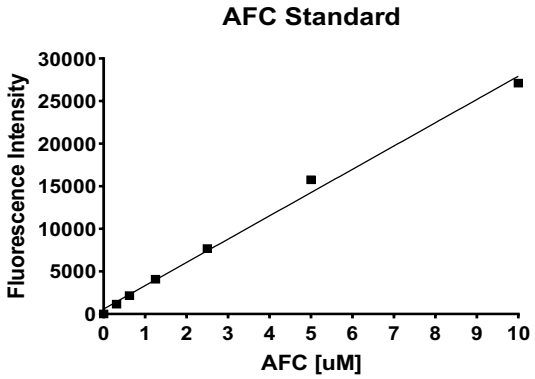
Materials needed but not supplied

1. Microplate reader
2. Adjustable micro-pipettor
3. Sterile Tips

Assay protocol

A. Making AFC standard curve

1. Dilute substrate solution to 20 µM with the assay buffer.
2. Dilute 1 mM AFC to 20 µM with the assay buffer.
3. Make 2-fold series of dilutions of AFC to 20, 10, 5, 2.5 1.25, 0.625, and 0 µM solutions with the 20 µM substrate solution from step 1.
4. Aliquot 50 µL of the diluted AFC solution to each well (96-well plate).
5. Add 50 µl of the assay buffer to each well to make 10, 5, 2.5 1.25, 0.625 and 0.3125 µM AFC solution.
6. Measure fluorescent intensity at excitation of 365 nm and emission of 505 nm.
7. Use the same machine settings when measure EK protease activity afterwards.



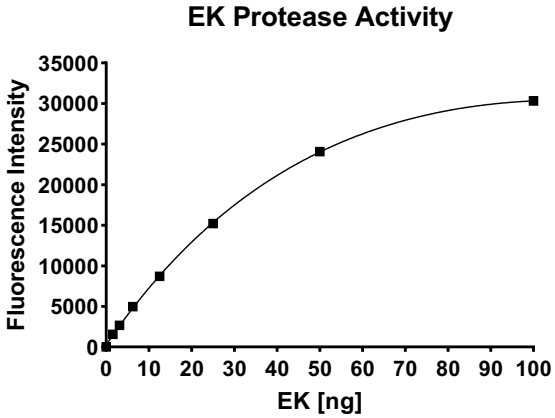
B. Measure EK positive control activity

1. Thaw EK protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: EK protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. **Do not re-use the diluted protein.**
2. Dilute the EK protein 100-fold with the assay buffer (from 400 ng/µL to 4 ng/µL). Then, make a further dilution to 2, 1, 0.5, 0.25, 0.125, and 0.0625 ng/µL.
3. Add 50 µl of diluted protein solution to each well (Test amount of the protein will be 200, 100, 50, 25, 12.5, 6.25, 3.125 ng per reaction).
4. Add 50 µl of assay buffer to negative control well(s).

   We recommend to run the reaction in duplicate.

5. Dilute substrate solution to 20 µM with assay buffer.
6. Add 50 µl of diluted substrate to each well.
7. Incubate at room temperature for 1 hour.
8. Measure fluorescent intensity at excitation of 365 nm and emission of 510 nm.
9. Plot fluorescent intensity versus protein concentration on a graph as below (subtract the average fluorescent intensity readings in the 0 ng wells from all of other wells to remove fluorescence background).



C. Measure EK protease activity

1. Dilute EK protease protein to 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 ng/µL with the assay buffer.
2. Add 50 µl of diluted protein solution to each well (Test amount of the protein will be 200, 100, 50, 25, 12.5, 6.25 and 3.125 ng per reaction).
3. Add 50 µl of assay buffer to negative control well(s).
   We recommend to run the reactions in duplicate.
4. Dilute substrate solution to 20 µM with assay buffer.
5. Add 50 µl of diluted substrate to each well.
6. Incubate at room temperature for 1 hour.
7. Measure fluorescent intensity at excitation of 465 nm and emission of 510 nm.
8. Plot fluorescent intensity versus protein concentration on a graph. (subtract the average fluorescent intensity readings in the 0 ng wells from all of other wells to remove fluorescence background).