Trypsin Activity Assay Kit (Fluorometric)

10/19

(Catalog # K2023-100; 100 assays; Store at -20°C)

I. Introduction:

Trypsin (EC 3.4.21.4) is a serine protease that is expressed in bacteria, fungi and mammals. Trypsin cleaves peptide chains at the carboxyl side of the amino acids namely arginine and lysine. In mammals, trypsin is secreted from the pancreas as an inactive precursor (trypsinogen) and is processed to its active form in the small intestine, where it plays an essential role in protein hydrolysis and absorption. Activation of trypsin in pancreas can lead to a series of events thereby resulting in pancreatitis. Trypsin is therefore a useful biomarker for pancreatitis. Additionally, monitoring trypsin activity plays a central role in biochemical, pharmaceutical and has clinical applications. BioVision's Trypsin Activity Assay Kit provides a simple, rapid way to detect trypsin activity in a wide variety of biological samples. In this kit, trypsin cleaves a synthetic substrate thereby releasing a fluorophore (AMC), which can be easily quantified at Ex/Em = 380/460 nm. By pretreating samples at 60ºC for 20 min, the kit can differentiate the trypsin activity from other structurally similar enzymes such as chymotrypsin and tryptase. The assay is specific, sensitive and can detect as low as 2 µU of trypsin activity in samples.

Substrate-AMC $\xrightarrow{\textbf{Trypsin}}$ Cleaved Substrate + AMC **(Ex/Em = 380/460 nm)**

II. Applications:

- Measurement of trypsin activity in Biological Fluids, Tissues.

III. Sample Type:

- Biological Fluids: Serum
- Tissue Homogenates: Intestine

IV. Kit Contents:

Components	K2023-100	Cap Code	Part Number
Trypsin Assay Buffer	35 ml	NM	K2023-100-1
Trypsin Substrate	50 µl	Red	K2023-100-2
Positive Control (lyophilized)	1 vial	Blue	K2023-100-3
AMC Standard	100 µl	Yellow	K2023-100-4
Trypsin Inhibitor	500 µl	Purple	K2023-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Dounce Tissue Homogenizer (BioVision Cat. #1998)
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **Trypsin Assay Buffer:** Store at either 4ºC or -20ºC. Bring to room temperature (RT) before use.
- **Trypsin Substrate:** Store at -20°C. Protect from light. Bring to RT before use.
- **Positive Control (lyophilized):** Reconstitute with 100 µl Trypsin Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use.
- **AMC Standard (1 mM in DMSO):** Store at -20ºC. Bring to RT before use.
- **Trypsin Inhibitor (TLCK, 20 mM):** Store at -20ºC. Bring to RT before use.

VII. Trypsin Activity Assay Protocol:

1. Sample Preparation:

Serum Sample(s): Clarify Serum Sample by centrifugation at 1,000 x *g* and 4ºC for 10 min in order to reduce the turbidity and separate the insoluble materials. Prepare a 20-fold dilution of the Serum Sample in Trypsin Assay Buffer (10 µl of Serum with 190 µl of Trypsin Assay Buffer) and incubate at 60ºC for 20 min. Centrifuge at 10,000 x *g* and 4ºC for 10 min and transfer the supernatant to a fresh tube. Add 40-70 µl of the diluted Sample into two parallel wells of a 96-well clear plate labeled as **"Sample"** and **"Sample Background Control"**. Add 10 µl of DMSO into the Sample well and 10 µl of Trypsin Inhibitor into the Sample Background Control well respectively. Adjust the volume of the Sample and Sample Background Control well to 80 µl/well with Trypsin Assay Buffer. Incubate at RT for 10 min in dark.

Tissue Sample(s): Add 250 µl of ice-cold Trypsin Assay Buffer to 50 mg of the Tissue Sample (wet weight). Homogenize on ice using a Dounce Tissue homogenizer (BioVision Cat. # 1998). Centrifuge the Sample(s) at 12,000 x *g* and 4ºC for 10 min. Collect the supernatant. Incubate the supernatant at 60ºC for 20 min. Centrifuge at 10,000 x *g* at 4ºC for 10 min and collect the Sample supernatant. Add 2-20 µl of the Sample supernatant into well(s) of a 96-well clear plate labeled as **"Sample"**. Prepare an additional well with the same volume of Trypsin Assay Buffer labeled as **"Background Control"** by adding 80 µl of Trypsin Assay Buffer. Adjust the volume of the Sample well(s) to 80 µl/well with Trypsin Assay Buffer.

Positive Control: Prepare an 800-fold dilution of the Positive Control (1 µl of Positive Control and 799 µl of Trypsin Assay Buffer). Add 2-6 µl of the diluted Positive Control into desired wells(s). Adjust the volume of the Positive Control well to 80 µl/well with Trypsin Assay Buffer.

Note:

For Unknown Samples, we recommend doing a pilot experiment and testing several doses to ensure that the readings are within the linear range of the Standard Curve.

2. **Standard Curve Preparation:** Prepare 0.1 mM AMC Standard solution by diluting the 1 mM stock AMC Standard (10 µl of 1 mM stock AMC Standard and 90 µl of Trypsin Assay Buffer). Add 0, 2, 4, 6, 8, 10 µl of 0.1 mM (0.1 nmol/µl) AMC Standard solution into a series of wells of a 96-well clear plate to generate 0, 0.2, 0.4, 0.6, 0.8, 1 nmol/well of AMC Standard respectively. Adjust the volume of each well to 100 µl with Trypsin Assay Buffer.
3. **Substrate Mix Preparation:** Prepare a 40-fold dilution of the Trypsin Substrate stock solution (2 µl of Trypsin Substrate and 78 µl Trypsin Assay Buffer resp.), vortex briefly. Add 20 µl of the diluted Trypsin Substrate solution to each well containing Sample(s), Positive Control and Background Control wells. The total volume in every well including Standards, Sample(s), Positive Control & Background Controls should be 100 µl.
4. **Measurement:** Measure the fluorescence intensity (Ex/Em = 380/460 nm) in kinetic mode for 10-60 min at RT using a fluorescence microtiter plate reader. Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding RFU for all Samples (R_{S1} and R_{S2}) and Background Controls (R_{B1} and R_{B2}). The AMC Standard Curve can be read in endpoint mode.

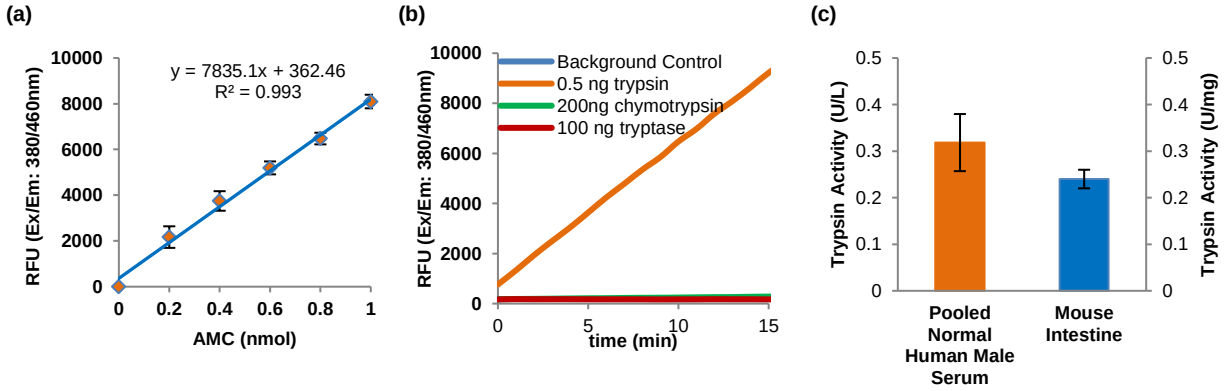
   Note: Shake the microplate carefully for 5 sec to mix the contents prior to reading the plate.
5. **Calculation:** Subtract 0 Standard RFU reading from all Standard readings. Plot the AMC Standard Curve and obtain the slope of the curve (**RFU/nmol**). Apply Samples ΔRFU (R_{S2}-R_{S1}) and Sample Background Controls ΔRFU (R_{B2}-R_{B1}) for serum samples or Samples ΔRFU (R_{S2}-R_{S1}) and Background Control ΔRFU (R_{B2}-R_{B1}) for tissue samples to the AMC Standard Curve to obtain the corresponding amount of AMC formed during the reaction time ($\Delta t = t_2$-t_1). Calculate the Background-corrected Sample (B, in nmol) by subtracting the amount of AMC formed by Sample Background Control and/or Background Control from the amount of AMC formed by Sample.

Calculate Trypsin Activity in the Sample as:

$$\textbf{Sample Trypsin Activity} = \frac{\textbf{B Sample(corrected)}}{\Delta t \cdot V \cdot P} * \textbf{D= nmol/min/mg = mU/mg}$$

Where: **B** is background-corrected AMC amount from the AMC Standard Curve (nmol)
Δt is the Reaction time (t_2-t_1 in min)
V is the Sample volume added into the reaction well (ml)
P is the initial protein concentration (mg/ml)
D is the Dilution factor (D = 1 for undiluted Samples)

Unit Definition: One unit of Trypsin activity is the amount of enzyme that generates 1.0 µmol of AMC per min at pH 8.0 at RT.



Figures: (a) AMC Standard Curve. **(b)** Measurement of Trypsin (0.5 ng), Chymotrypsin (200 ng) and Tryptase (100 ng) activities. Samples were pretreated at 60ºC for 20 min. **(c)** Measurement of Trypsin activity in pooled normal human male Serum (1:20 dilution, 50 µl) and mouse Intestine extracts (0.4 µg protein). All assays were performed following the kit protocol.

VIII. Related Products:

Chymotrypsin Activity Assay Kit (Fluorometric) (K352)
Trypsin Activity Colorimetric Assay Kit (K771)
Trypsin, Human Pancreas (7292)
Trypsin, Recombinant (P1228)
Alpha 2 Antiplasmin, Human Plasma (7295)
Trypsin (Mouse) ELISA Kit (E4362)
Pepsin/Pepsinogen Activity Assay Kit (Fluorometric) (K446)
Protease Activity Fluorometric Assay Kit (K781)
Trypsin Substrate (Colorimetric) (B2451)
Chymotrypsin, Human Pancreas (7538)
Trypsin (Human) ELISA Kit (E4361)
Trypsin (Rat) ELISA Kit (E4363)

FOR RESEARCH USE ONLY! Not to be used on humans