Human Urinary Trypsin Inhibitor ELISA

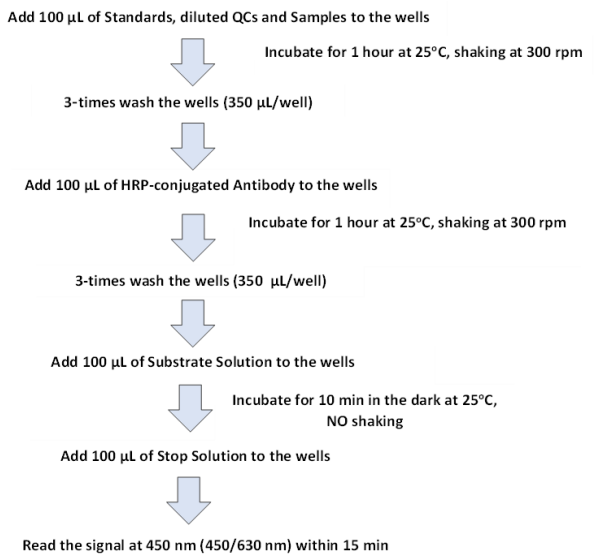
Cat. No.: BA3010

Enzyme Immunoassay for the quantitative determination Urinary Trypsin Inhibitor (UTI) in human urine.

Urinary trypsin inhibitor (UTI) (also called bikunin [1] or ulinastatin [2]) is a multivalent serine protease inhibitor synthesized and released in human urine and blood [3].
UTI is a positive acute phase protein [13]. The concentration of free, uncomplexed UTI in plasma of patients with inflammatory conditions has been reported to be higher than normal [12, 14, 15]. The plasma UTI level and its gene expression change under severe inflammatory conditions [16]. In patients suffering from various nephropathies, a clear correlation between the UTI and creatinine concentrations in plasma was found [17], implying that the kidneys are a major site of uptake of the protein [13]. UTI is rapidly released into urine when infection occurs and is an excellent inflammatory marker, constituting most of the urinary anti-trypsin activity [18]. In urine, in which the level of complexed UTI is negligible, the average UTI concentration is 0.03 – 0.05 µM [10, 19].
The level of UTI in urine may be elevated under various pathological conditions, including pneumonia [20], lung emphysema [21], rheumatoid arthritis [22], cancer [23], and surgical trauma [24].

PRINCIPLE OF URINARY TRYPSIN INHIBITOR ELISA

The microtiter plate is coated with the antibody specifically binding the Urinary Trypsin Inhibitor. The human urine is incubated in the plate with the capture antibody.
The specimen is washed out and the specifically bound protein is incubated with HRP-labelled detection antibody. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).
The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of UTI in the specimen. The concentration of UTI in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	***Qty.***
Antibody Coated Microtiter Plate	96 wells
Antibody-HRP Conjugate	13 mL
Master Standard (lyophilized)	2 vial
Dilution Buffer 5x conc.	2x13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ±2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ±2°C.

Preparation of Standards

Reconstitute lyophilized Human UTI Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human UTI in Master Standard is 20 ng/mL,

Prepare set of Standard solution as follows:

Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 20 ng/mL (lyophilised)	See CofA	20 ng/mL
Std2	300 µL of Std1	300 µL	10 ng/mL
Std3	300 µL of Std2	300 µL	5 ng/mL
Std4	300 µL of Std3	300 µL	2.5 ng/mL
Std5	300 µL of Std4	300 µL	1.25 ng/mL
Std6	300 µL of Std5	300 µL	0.63 ng/mL
Blank	-	200 µL	0 ng/mL

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of Dilution Buffer 1x

Prepare a working solution of Dilution Buffer by mixing 13 mL (26 mL) of Dilution Buffer 5x conc. and 52 mL (104 mL) of deionized/ distilled water (dH_2O). Prepare only the amount for immediate consumption. Mix well. Store at 4°C for two weeks.

Preparation of samples

Human urine may be used with this assay. It is recommended to assay not-frozen samples. For long-term storage the urine samples should be frozen in protective medium at minimum -70°C.

Recommended dilution of samples is 1: 400. It is recommended to use the two-step dilution.
Dilution A (20x) for both singlets and duplicates: 10 µL of samples + 190 µL of Dilution Buffer.
Dilution B (20x): 20 µL of Dilution A + 380 µL of Dilution Buffer, for both singlets and duplicates.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL Substrate solution, incubate for **10 min,** at 25°C ±2°C**.** Avoid exposure to the light during this step.
7. Pipette 100 µL of STOP solution.

Read the signal at 450 or 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:400 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human UTI giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.254 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (µg/mL)	SD	CV (%)
1	0.75	0.04	5.0
2	4.68	0.17	3.6

Inter-assay (Run – to – run)

Sample	Mean (µg/mL)	SD	CV (%)
1	0.66	0.07	10.8
2	4.77	0,30	6.2

3. Accuracy

Dilution linearity

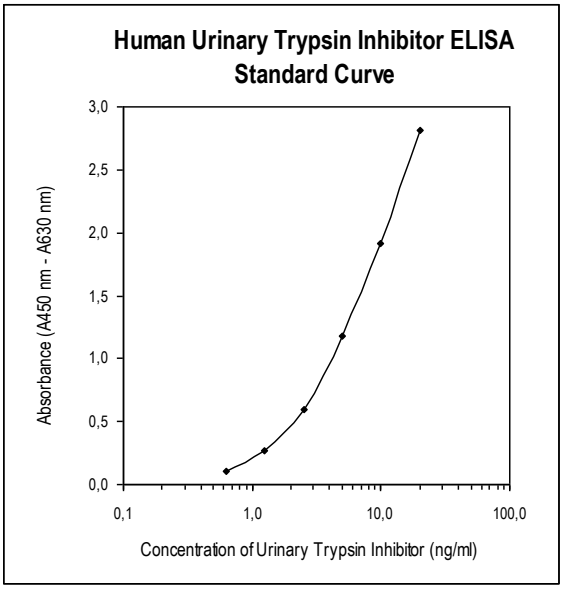
Sample	Dilution	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
1		4.66	-	-
	2x	2.40	2.33	103
	4x	1.22	1.16	105
	8x	0.62	0.58	106
2		3.60	-	-
	2x	1.78	1.80	99
	4x	0.94	0.90	105
	8x	0.51	0.45	113

Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
1	-	1.22	-	-
	0.5	1.84	1.72	107
	1.0	2.28	2.22	103
	2.0	3.11	3.22	97

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

[1] Słota A, Sjöquist M, Wolgast M, Alston-Smith J, Fries E. Bikunin in rat plasma, lymph and bile. Biol Chem Hoppe Seyler; 375(2):127-133. (1994)
[2] Odum L, Hansen-Nord G, Byrjalsen I. Human inter-alpha-trypsin inhibitor and immunologically related inhibitors investigated by quantitative immunoelectrophoresis. II. Pathological conditions. Clin Chim Acta; 162(2):189-198. (1987)
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[4] Fries E, Blom AM. Bikunin--not just a plasma proteinase inhibitor. Int J Biochem Cell Biol; 32(2):125-137. (2000)
[5] Sugiki M, Maruyama M, Yoshida E, Sumi H, Mihara H. Acid-stable protease inhibitor in chronic phase of carrageenin-induced inflammation in rats. Inflammation; 15(4):281-289. (1991)