**Quantitative Determination of Human Urinary Trypsin Inhibitor**

**Human Urinary Trypsin Inhibitor ELISA**

- Sensitivity (0.254 ng/ml)
- Excellent analytical characteristics
- Validated for human urine samples
- Preliminary population data

**Infection and Inflammation**
- Sepsis
- Renal Disease
- Oncology

**New Product**
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 an acidic glycoprotein, composed of 143-amino acid residues. Bikunin contains two proteinase inhibitor domains of the Kunitz type [4], a short connecting peptide as well as N- and C-terminal extensions 10 - 25 amino acids long. The N-terminal extension carries a chondroitin sulphate chain [5]. Each of the Kunitz domains has a binding site for a proteinase [6] and the amino acid residues essential for binding (Met 36 of the N-terminal domain and Arg 92 of the C-terminal domain). The total molecular weight of UTI is 25 - 26 kDa.

UTI is produced by the endoplasmic reticulum of hepatocytes as a precursor in which UTI is linked to α₁-microglobulin [7, 8]. Most of the UTI in blood (90 - 98 %) occurs as a covalently linked subunit of the proteins pre-α-inhibitor and inter-α-inhibitor, respectively [9, 10]. In human plasma the major UTI-containing protein is inter-α-inhibitor [11]. The total concentration of UTI in human plasma is 4 - 7 μM [10, 12], of which 2 - 10 % is in free form [9, 10].

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]. It appears that UTI passes through the kidneys by glomerular filtration [25]. Some tumour cells secrete UTI [26], which could contribute to the high urinary levels seen in some cancer patients [13].

The function of UTI has been preserved during evolution [27]. Trypsin and other serine proteases such as trombin, chymotrypsin, kallikrein [28], plasmin [29], neutrophil elastase, cathepsin and factors IXa, Xa [28], Xla and Xlla are inhibited by UTI [30, 31], indicating that UTI is part of the inflammatory process. Furthermore, UTI can suppress urokinase-type plasminogen activator (uPA) expression through the inhibition of protein kinase C [32, 33]. UTI appears to prevent organ injury by inhibiting the activity of
**Intended use**

The RD191343100R Human Urinary Trypsin Inhibitor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human urinary trypsin inhibitor.

- The total assay time is less than 3 hours
- The kit measures urinary trypsin inhibitor in human urine
- Assay format is 96 wells
- Standard is purified human native protein
- Components of the kit are provided ready to use, concentrated or lyophilized

**Clinical application**

- Infection and Inflammation
- Sepsis
- Renal disease
- Oncology

**Test principle**

In the BioVendor Human Urinary Trypsin Inhibitor ELISA, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-human urinary trypsin inhibitor antibody. After 60 minutes incubation and washing, polyclonal antibody against human urinary trypsin inhibitor conjugated with horseradish peroxidase (HRP) is added into the wells and incubated with the captured urinary trypsin inhibitor for 60 minutes. Following the last washing step, the bound conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by the addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of urinary trypsin inhibitor. A standard curve is constructed by plotting absorbance values against urinary trypsin inhibitor concentrations of Standards and concentrations of unknown samples are determined using this standard curve.

**Clinical application**

Inflammatory serine proteases [34, 35]. *In vitro* studies have demonstrated that serine protease inhibitors may have anti-inflammatory properties [27]. UTI suppresses the infiltration of neutrophils and the release of elastase and chemical mediators from them [36-38].

Clinically, UTI is widely used as a drug for patients with acute inflammatory disorders such as pancreatitis, shock and disseminated intravascular coagulation [27].

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**BioVendors Human Urinary Trypsin Inhibitor ELISA (RD191343100R)**

**HUMAN URINARY TRYSIN INHIBITOR ELISA**

CAT. NO.: RD191343100R

<table>
<thead>
<tr>
<th>Assay format</th>
<th>Sandwich ELISA, HRP-labelled antibody, 96 wells/kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Urine</td>
</tr>
<tr>
<td>Standards</td>
<td>0.63 to 20 ng/ml</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.254 ng/ml</td>
</tr>
</tbody>
</table>

**Absorbance at 450 nm**

![Graph showing absorbance at 450 nm vs. Hu Urinary Trypsin Inhibitor (ng/ml)](image-url)
**HUMAN URINARY TRYPSIN INHIBITOR ELISA**

### Precision

#### Intra-assay (Within-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (µg/ml)</th>
<th>SD (µg/ml)</th>
<th>CV (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.75</td>
<td>0.04</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>4.68</td>
<td>0.17</td>
<td>3.6</td>
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</table>

#### Inter-assay (Run-to-Run) (n=5)

<table>
<thead>
<tr>
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<th>Mean (µg/ml)</th>
<th>SD (µg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.66</td>
<td>0.07</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>4.77</td>
<td>0.30</td>
<td>6.2</td>
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</table>

### Spiking recovery

Urine samples were spiked with different amounts of human urinary trypsin inhibitor and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Recovery O/E (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.71</td>
<td>-</td>
<td>95.1</td>
</tr>
<tr>
<td></td>
<td>0.91</td>
<td>0.96</td>
<td>90.6</td>
</tr>
<tr>
<td></td>
<td>1.09</td>
<td>1.21</td>
<td>92.8</td>
</tr>
<tr>
<td></td>
<td>1.58</td>
<td>1.71</td>
<td>92.8</td>
</tr>
<tr>
<td>2</td>
<td>1.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.84</td>
<td>1.72</td>
<td>107.1</td>
</tr>
<tr>
<td></td>
<td>2.28</td>
<td>2.22</td>
<td>103.0</td>
</tr>
<tr>
<td></td>
<td>3.11</td>
<td>3.22</td>
<td>96.8</td>
</tr>
</tbody>
</table>

### Linearity

Urine samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>4.66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>2.40</td>
<td>2.33</td>
<td>103.3</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>1.22</td>
<td>1.16</td>
<td>105.2</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>0.62</td>
<td>0.58</td>
<td>105.8</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>3.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1.78</td>
<td>1.80</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>0.94</td>
<td>0.90</td>
<td>105.0</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>0.51</td>
<td>0.45</td>
<td>113.0</td>
</tr>
</tbody>
</table>

### Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples (400x)
- Add 100 µl Standards and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Conjugate Solution
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl Stop Solution
- Read absorbance and calculate results
Preliminary Population Data

The range of the urine samples from healthy donors (n = 31) was determined using this Human Urinary Trypsin Inhibitor ELISA kit in our laboratory: Mean concentration of urinary trypsin inhibitor from this healthy donors was 4.17 μg/ml (median = 2.85 μg/ml, SD = 3.81).

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for urinary trypsin inhibitor levels with the assay.

Related products

- RD181343100 Urinary Trypsin Inhibitor Human, Rabbit Polyclonal Antibody
References


