Human Tissue Plasminogen Activator ELISA Kit
ab190812
SimpleStep ELISA

Overview

Product name: Human Tissue Plasminogen Activator ELISA Kit
Detection method: Colorimetric

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td>5</td>
<td>3.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td>3</td>
<td>10.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample type: Cell culture supernatant, Serum, Heparin Plasma, EDTA Plasma, Citrate Plasma
Assay type: Sandwich (quantitative)
Sensitivity: 3.5 pg/ml
Range: 78 pg/ml - 5000 pg/ml

Recovery

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>104</td>
<td>100% - 107%</td>
</tr>
<tr>
<td>Cell culture media</td>
<td>119</td>
<td>116% - 123%</td>
</tr>
<tr>
<td>Heparin Plasma</td>
<td>97</td>
<td>94% - 99%</td>
</tr>
<tr>
<td>EDTA Plasma</td>
<td>84</td>
<td>82% - 86%</td>
</tr>
<tr>
<td>Citrate Plasma</td>
<td>99</td>
<td>97% - 107%</td>
</tr>
</tbody>
</table>

Sample specific recovery
**Assay time**
1h 30m

**Assay duration**
One step assay

**Species reactivity**
React with: Human
Does not react with: Mouse, Rat, Rabbit, Goat, Guinea pig, Hamster, Cow, Dog, Pig

**Product overview**
Tissue Plasminogen Activator *in vitro* SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Tissue Plasminogen Activator (tPA) protein in human serum, plasma and cell culture supernatant samples.

The SimpleStep ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

**Notes**
Tissue Plasminogen Activator (Tissue-type plasminogen activator or tPA) is a circulating serine protease involved in the breakdown of clots. tPA converts inactive plasminogen to active plasmin; in turn plasmin degrades the fibrin matrix in clots. In addition, plasmin can cleave tPA at Arg-310 with results in a two chain disulphide linked tPA that has even greater proteolytic activity. tPA is synthesized in many tissues and is secreted into most extracellular body fluids. Recombinant tPA is used medically to resolve or prevent blood clots in ischemic stroke or myocardial infarction.

**Tested applications**
Suitable for: Sandwich ELISA

**Platform**
Microplate

**Properties**

**Storage instructions**
Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X TPA Capture Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>10X TPA Detector Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>10X Wash Buffer PT (ab206977)</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>ab221824 - Antibody Diluent 4BI</td>
<td>1 x 6ml</td>
</tr>
<tr>
<td>Plate Seals</td>
<td>1 unit</td>
</tr>
<tr>
<td>Sample Diluent NS (ab193972)</td>
<td>1 x 50ml</td>
</tr>
<tr>
<td>SimpleStep Pre-Coated 96-Well Microplate (ab206978)</td>
<td>1 unit</td>
</tr>
</tbody>
</table>
**Function**
Converts the abundant, but inactive, zymogen plasminogen to plasmin by hydrolyzing a single Arg-Val bond in plasminogen. By controlling plasmin-mediated proteolysis, it plays an important role in tissue remodeling and degradation, in cell migration and many other physiopathological events. Play a direct role in facilitating neuronal migration.

**Tissue specificity**
Synthesized in numerous tissues (including tumors) and secreted into most extracellular body fluids, such as plasma, uterine fluid, saliva, gingival crevicular fluid, tears, seminal fluid, and milk.

**Involvement in disease**
Note=Increased activity of TPA results in increased fibrinolysis of fibrin blood clots that is associated with excessive bleeding. Defective release of TPA results in hypofibrinolysis that can lead to thrombosis or embolism.

**Sequence similarities**
Belongs to the peptidase S1 family.
Contains 1 EGF-like domain.
Contains 1 fibronectin type-I domain.
Contains 2 kringle domains.
Contains 1 peptidase S1 domain.

**Domain**
Both FN1 and one of the kringle domains are required for binding to fibrin.
Both FN1 and EGF-like domains are important for binding to LRP1.
The FN1 domain mediates binding to annexin A2.
The second kringle domain is implicated in binding to cytokeratin-8 and to the endothelial cell surface binding site.

**Post-translational modifications**
The single chain, almost fully active enzyme, can be further processed into a two-chain fully active form by a cleavage after Arg-310 catalyzed by plasmin, tissue kallikrein or factor Xa.
Differential cell-specific N-linked glycosylation gives rise to two glycoforms, type I (glycosylated at Asn-219) and type II (not glycosylated at Asn-219). The single chain type I glycoform is less readily converted into the two-chain form by plasmin, and the two-chain type I glycoform has a lower activity than the two-chain type II glycoform in the presence of fibrin.
N-glycosylation of Asn-152; the bound oligomannosidic glycan is involved in the interaction with the mannose receptor.
Characterization of O-linked glycan was studied in Bowes melanoma cell line.

**Cellular localization**
Secreted > extracellular space.

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**Applications**

Our Abpromise guarantee covers the use of ab190812 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwich ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>
Background-subtracted data values (mean +/- SD) are graphed.

Example of TPA standard curve

Background-subtracted data values (mean +/- SD, n=2) are graphed.

Titration of Human serum and plasma (heparin) within the working range of the assay

PBMC were grown in the absence or presence of phytohemagglutinin (PHA) for 2 days. TPA was measured in 4-fold diluted cell culture supernatants of unstimulated and PHA stimulated PBMC. Measured values were interpolated from the TPA Standard Curve diluted in Sample Diluent NS and DF corrected. Mean +/- SD, n=2, are graphed.

Comparison of secreted TPA in unstimulated and PHA-stimulated Human PBMC
Serum from 10 apparently healthy male donors was measured in duplicate. The mean TPA concentration was determined to be 2,741 pg/mL with a range of 1,836-4,012 pg/mL in male donors.

The concentrations of TPA were measured in duplicate and interpolated from the TPA standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TPA concentration was determined to be 4,710 pg/mL in serum and 4,270 pg/mL in plasma (heparin).

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