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# ab220653
# Human Tissue Factor SimpleStep ELISA® Kit

For the quantitative measurement of human Tissue Factor in plasma, saliva, cell culture supernatants, cell extracts, and urine.

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

# 1. Overview

Tissue factor in vitro SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Tissue Factor protein in plasma, saliva, cell culture supernatants, cell extracts, and urine.

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 Development Solution 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.

Tissue factor (TF), also known as Coagulation factor III, F3 thromboplastin, and CD142 is a 263-amino acid single pass type I membrane protein encoded by the gene F3. TF is a 47 kDa single pass type I membrane protein consisting of an extracellular, transmembrane, and cytoplasmic domain. Tissue factor's main role is in blood coagulation. The protein acts as a receptor for coagulation factor VII, which then forms the active complex FVIIa. This complex proteolytically activates downstream factors, including coagulation Factor IX and X, which leads to the formation of thrombin and fibrin clot formation. Tissue Factor is in a class of proteins known as Cytokine Receptor class II family. This family is activated by cytokines, and play a role in angiogenesis and apoptosis.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

## 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

## 4. Storage and Stability

**Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Human Tissue Factor Capture Antibody 10X | 600 µL | +4°C |
| Human Tissue Factor Detector Antibody 10X | 600 µL | +4°C |
| Human Tissue Factor Lyophilized Recombinant Protein | 2 Vials | +4°C |
| Antibody Diluent 4BI | 6 mL | +4°C |
| Wash Buffer PT 10X | 20 mL | +4°C |
| Cell Extraction Buffer PTR 5X | 10 mL | +4°C |
| Cell Extraction Enhancer Solution 50X | 1 mL | +4°C |
| TMB Development Solution | 12 mL | +4°C |
| Stop Solution | 12 mL | +4°C |
| Sample Diluent NS | 50mL | +4°C |
| Anti-tag coated microplate (12 x 8 well strips) | 96 Wells | +4°C |
| Plate Seal | 1 | +4°C |

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
-

## 8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

### 9.1 1X Cell Extraction Buffer PTR (For cell extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

### 9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

### 9.3 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

## 10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

**10.1** **IMPORTANT**: If the protein standard vial has a volume identified on the label, reconstitute the human Tissue Factor protein standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the human Tissue Factor protein standard by adding 500 µL Diluent.

For **cell extract samples measurements**, reconstitute the human Tissue Factor protein standard by adding 1X Cell Extraction Buffer PTR.

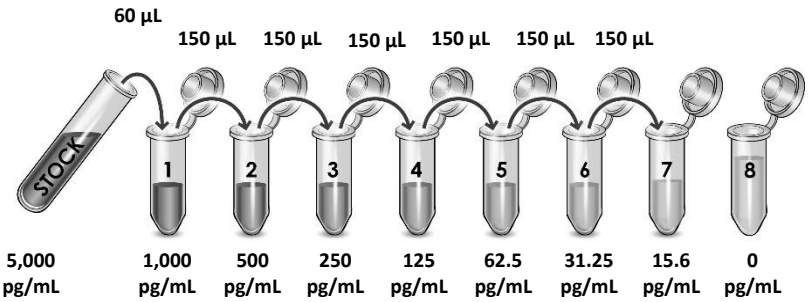
For **plasma, cell culture supernatants, urine, and saliva samples measurements,** reconstitute the human Tissue Factor protein standard by adding Sample Diluent NS.

Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 5,000 pg/mL **Stock Standard** Solution.

10.1.1 Label eight tubes, Standards 1– 8.

10.1.2 Add 240 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.

10.1.3 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



# 11. Sample Preparation

| Typical Sample Dynamic Range | |
|---|---|
| **Sample Type** | **Range** |
| Human Plasma - Citrate | 12.5 -100% |
| Human Plasma – EDTA | 12.5 -100% |
| Human Plasma - Heparin | 12.5 -100% |
| Human Saliva | 3.1-50% |
| Human Urine | 0.78-6.25% |
| A431 cell culture supernatant | 6.25-100% |
| A431 Cell Extract | 0.16-5 µg/mL |
| U-87 Cell Extract | 1.5-100 µg/mL |

### 11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay OR dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

### 11.2 Urine:

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

### 11.3 Saliva:

Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants and assay OR Dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

### 11.4 Preparation of extracts from cell pellets:

11.4.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.4.2 Rinse cells twice with PBS.

11.4.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.4.4 Incubate on ice for 20 minutes.

11.4.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.4.6 Transfer the supernatants into clean tubes and discard the pellets.

11.4.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.4.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

### 11.5 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.5.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.5.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.5.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.5.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.5 Transfer the supernatants into clean tubes and discard the pellets.

11.5.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## 12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

## 13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

**13.1** Prepare all reagents, working standards, and samples as directed in the previous sections.

**13.2** Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

**13.3** Add 50 µL of all sample or standard to appropriate wells.

**13.4** Add 50 µL of the Antibody Cocktail to each well.

**13.5** Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

**13.6** Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

**13.7** Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
*Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*
*Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*

**13.8** Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

**13.9** Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

| Mode | Kinetic |
|---|---|
| Wavelength: | 600 nm |
| Time: | up to 20 min |
| Interval: | 20 sec - 1 min |
| Shaking: | Shake between readings |

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

**13.10** Analyze the data as described below.

## 14. Calculations

**14.1** Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

**14.2** **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

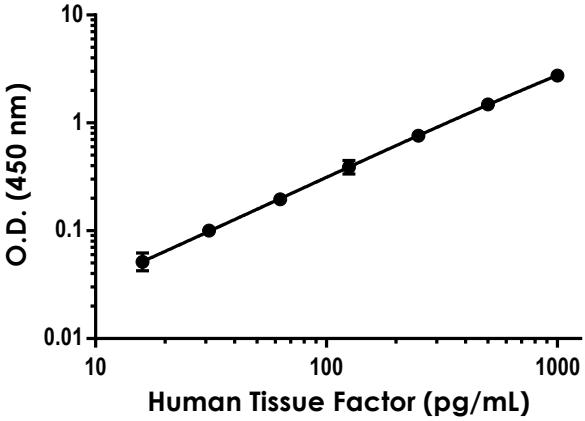
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

**14.3** Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

**14.4** Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

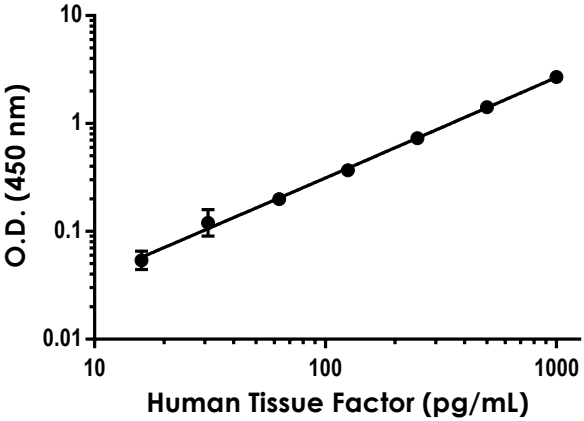
## 15. Typical Data

**Typical standard curve** – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.




| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.061 | 0.065 | 0.063 |
| 15.63 | 0.108 | 0.122 | 0.115 |
| 31.25 | 0.167 | 0.160 | 0.163 |
| 62.5 | 0.260 | 0.258 | 0.259 |
| 125 | 0.493 | 0.414 | 0.453 |
| 250 | 0.835 | 0.813 | 0.824 |
| 500 | 1.620 | 1.477 | 1.549 |
| 1,000 | 2.790 | 2.843 | 2.816 |

**Figure 1**. Example of human Tissue Factor standard curve in Sample Diluent NS. The Tissue Factor standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.




| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.058 | 0.062 | 0.060 |
| 15.63 | 0.122 | 0.107 | 0.114 |
| 31.25 | 0.206 | 0.158 | 0.182 |
| 62.5 | 0.276 | 0.243 | 0.260 |
| 125 | 0.434 | 0.421 | 0.428 |
| 250 | 0.814 | 0.765 | 0.790 |
| 500 | 1.495 | 1.452 | 1.474 |
| 1,000 | 2.789 | 2.724 | 2.756 |

**Figure 2**. Example of human Tissue Factor standard curve in 1X Cell Extraction Buffer PTR. The Tissue Factor standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

## 16. Typical Sample Values

**SENSITIVITY** –

The calculated minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

| Sample Diluent Buffer | n= | Minimal Detectable Dose |
|---|---|---|
| Sample Diluent NS | 8 | 3.6 pg/mL |
| 1X Cell Extraction Buffer PTR | 8 | 7.6 pg/mL |

**RECOVERY** –

Three concentrations of human Tissue Factor were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Human Plasma- Citrate 50% | 104 | 99-113 |
| Human Plasma-EDTA 50% | 115 | 111-118 |
| Human Plasma-Heparin 50% | 109 | 107-113 |
| Human Urine 25% | 83 | 80-85 |
| Human Saliva 50% | 86 | 83-87 |
| HGDMEM, 50% | 110 | 105-116 |
| U-87 cell extract, 6.25 µg/mL | 105 | 100-107 |

**Linearity of Dilution**

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Tissue Factor was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

| Dilution Factor | Interpolated value | 100% Human Plasma (Citrate) | 100% Human Plasma (EDTA) | 100% Human Plasma (Heparin) | 50% Saliva |
|---|---|---|---|---|---|
| Undiluted | pg/mL | 49.6 | 68.8 | 78.5 | 91.9 |
| | **% Expected value** | 100 | 100 | 100 | 100 |
| 2 | pg/mL | 27.1 | 29.4 | 40.7 | 100 |
| | **% Expected value** | 109 | 85 | 104 | 109 |
| 4 | pg/mL | 12.2 | 13.9 | 16.4 | 102 |
| | **% Expected value** | 99 | 81 | 84 | 111 |
| 8 | pg/mL | 5.9 | 10 | 10.8 | 87.5 |
| | **% Expected value** | 95 | 116 | 110 | 95 |

Recombinant human Tissue Factor was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

| Dilution Factor | Interpolated value | 50% Human Plasma (Citrate) | 50% Human Plasma (Heparin) | 50% Human Plasma (EDTA) | 50% Saliva | 6.25% Urine |
|---|---|---|---|---|---|---|
| Undiluted | pg/mL | 519 | 561 | 569 | 617 | 121 |
| | **% Expected value** | 100 | 100 | 100 | 100 | 100 |
| 2 | pg/mL | 251 | 261 | 260 | 246 | 63.5 |
| | **% Expected value** | 97 | 93 | 91 | 80 | 106 |
| 4 | pg/mL | 123 | 126 | 127 | 123 | 32 |
| | **% Expected value** | 95 | 90 | 89 | 80 | 107 |
| 8 | pg/mL | 72 | 39.3 | 77 | 75 | 14.2 |
| | **% Expected value** | 111 | 92 | 108 | 97 | 94 |
| 16 | pg/mL | 39 | 16 | 45 | 37 | NL |
| | **% Expected value** | 120 | 112 | 145 | 97 | - |

NL – Non-Linear

Native Tissue Factor was measured in the following biological samples in a 2-fold dilution series. Cellular extracts dilutions are made in 1X Cell Extraction Buffer. Cell culture supernatant dilutions are made in Sample Diluent NS

| Dilution Factor | Interpolated value | 100 µg/mL U-87 extract | 100% A431 CCS | 5 µg/mL A431 extract |
|---|---|---|---|---|
| Undiluted | pg/mL | 1018 | 91.1 | 531 |
| | **% Expected value** | 100 | 100 | 100 |
| 2 | pg/mL | 605 | 45.5 | 267 |
| | **% Expected value** | 119 | 100 | 101 |
| 4 | pg/mL | 273 | 25.3 | 129 |
| | **% Expected value** | 107 | 111 | 97 |
| 8 | pg/mL | 120 | 13.3 | 58 |
| | **% Expected value** | 94 | 117 | 88 |
| 16 | pg/mL | 65 | 6 | 28 |
| | **% Expected value** | 102 | 108 | 85 |

**PRECISION** –

Mean coefficient of variations of interpolated values from Tissue Factor within the working range of the assay.

| | Intra-Assay | Inter-Assay |
|---|---|---|
| n = | 8 | 3 |
| CV(%) | 4.9 | 6.9 |

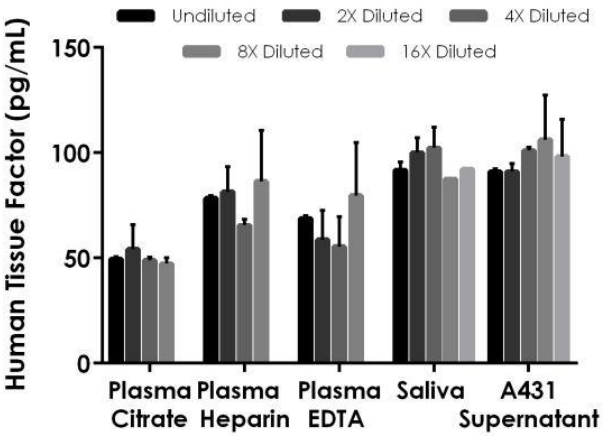



**Figure 3.** Interpolated concentrations of native Tissue Factor in human plasma and A431 cell culture supernatant samples. The concentrations of Tissue factor were measured in duplicates, interpolated from the Tissue Factor standard curves and corrected for sample dilution. Undiluted samples are as follows: plasma (citrate) 100%, plasma (heparin) 100%, plasma (EDTA) 100%, saliva 50% and A431 cell culture supernatant 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Tissue Factor concentration was determined to be 50 pg/mL in plasma (citrate), 78 pg/mL in plasma (heparin), 65.7 pg/mL in plasma (EDTA), 98 pg/mL in saliva) and 97.5 pg/mL in A431 cell culture supernatant.

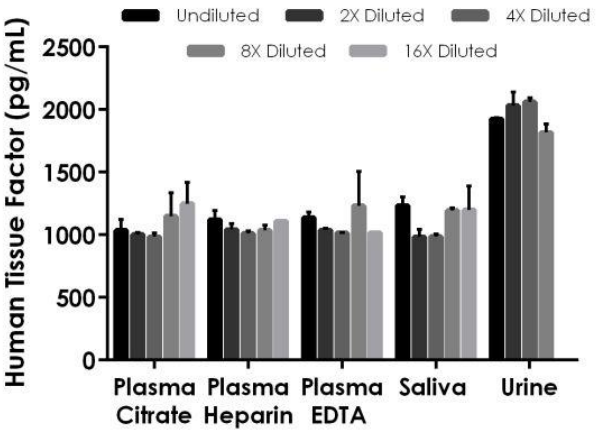



**Figure 4.** Interpolated concentrations of spiked Tissue Factor in human plasma, saliva, and urine samples. The concentrations of Tissue Factor were measured in duplicates, interpolated from the Tissue Factor standard curves and corrected for sample dilution. Undiluted samples are as follows: plasma (citrate) 50%, plasma (heparin) 50%, saliva 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Tissue Factor concentration was determined to be 1,000 pg/mL in neat plasma (citrate), plasma (heparin), plasma (EDTA), saliva and 2,000 pg/mL in neat urine.

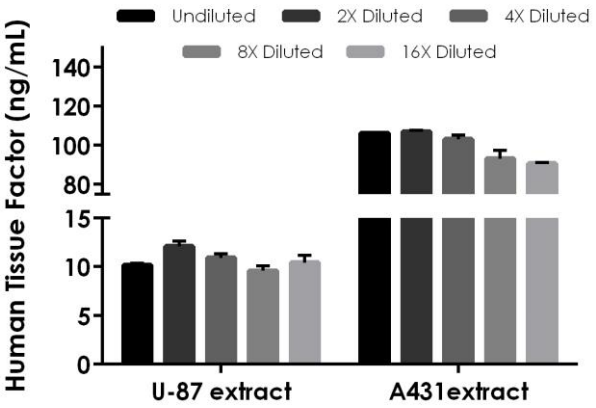



**Figure 5**. Interpolated concentrations of native Tissue Factor in U-87 and A431 cellular extract samples based on a 1,000 µg/mL extract load. The concentrations of Tissue Factor were measured in duplicate and interpolated from the Tissue Factor standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Tissue Factor concentration was determined to be 10.4 ng/mL in U-87 cell extract and 100 ng/mL in A431 cell extract.

## 17. Assay Specificity

This kit recognizes both native and recombinant Human Tissue Factor protein in plasma, saliva, urine, cell culture supernatant, and cell extract samples only.

This assay is specific to the full-length, extracellular portion of human Tissue Factor.

Tissue extract samples have not been tested with this kit.

## 18. Species Reactivity

This kit recognizes human Tissue Factor protein.

Other species reactivity was determined by measuring neat plasma samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human plasma assayed at the same dilution.

Reactivity < 1% was determined for the following species:

- Mouse
- Rat
- Rabbit
- Cow

Please contact our Technical Support team for more information.

## 19. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| **Difficulty pipetting lysate; viscous lysate.** | Genomic DNA solubilized | Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction. |
| **Poor standard curve** | Inaccurate Pipetting | Check pipettes |
| | Improper standard dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| **Low Signal** | Incubation times too brief | Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| | Incubation times with TMB too brief | Ensure sufficient incubation time until blue color develops prior addition of Stop solution |
| **Large CV** | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions. |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| **Low sensitivity** | Improper storage of the ELISA kit | Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light. |
| **Precipitate in Diluent** | Precipitation and/or coagulation of components within the Diluent. | Precipitate can be removed by gently warming the Diluent to 37°C. |

## 20. Notes

## Technical Support



**For all technical or commercial enquiries please go to:**

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)