## CATALOG NO: EK0771

## LOT NO: LOT#\_\_\_\_\_\_\_

## INTENDED USE

For the quantitation of Human SPINT1 concentrations in cell culture supernatants, serum and plasma (heparin).

## BACKGROUND

Kunitz-type protease inhibitor 1 is an enzyme that in humans is encoded by the SPINT1 gene. It is a member of the Kunitz family of serine protease inhibitors. The SPINT1 gene encodes an inhibitor of hepatocyte growth factor activator (HGFAC) and matriptase and is involved in degradation of the extracellular matrix. SPINT1 is produced as an active membrane-associated protein and is proteolytically cleaved and secreted. Alternative splicing results in multiple variants encoding different isoforms. Human SPINT1/HAI-1 ELISA Kit

## PRINCIPLE OF THE ASSAY

The Innovative Research Human SPINT1 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human SPINT1 with a 96-well strip plate that is pre-coated with antibody specific for SPINT1. The detection antibody is a biotinylated antibody specific for SPINT1. The kit includes Human SPINT1 protein as standards. To measure Human SPINT1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human SPINT1 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human SPINT1 in the sample.

## OVERVIEW

\*The sensitivity or the minimum detectable dose (MDD) is the lower limit of the target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

## TECHNICAL DETAILS

Product Name: Human SPINT1/HAI-1 ELISA Kit Reactive Species: Human Size: 96 wells/kit, with removable strips. Description: Human SPINT1/HAI-1 ELISA Kit (96 Tests). Quantitate Human SPINT1 in cell culture supernatants, serum and plasma (heparin). Sensitivity: 15pg/ml. The brand indicates this is a premium quality ELISA kit. Each kit delivers precise quantification, high sensitivity, and excellent reproducibility. Only our most reliable and effective kits qualify as , guaranteeing top-tier results for your assays. Sensitivity\*: 15 pg/ml Detection Range: 156 pg/ml - 10,000 pg/ml Storage Instructions: Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.) Uniprot ID: O43278 Specificity: Natural and recombinant Human SPINT1 Standard Protein: Expression system for standard: NS0; Immunogen sequence: G36-L529 Cross-reactivity: This kit is for the detection of Human SPINT1. No significant cross-reactivity or interference between SPINT1 and its analogs was observed. This claim is limited by existing techniques; therefore, cross-reactivity may exist with untested analogs.

## PREPARATIONS BEFORE ASSAY

Please read the following instructions before starting the experiment. Read this manual in its entirety in order to minimize the chance of error. Confirm that you have the appropriate non-supplied equipment available. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation). To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended. Before using the kit, spin tubes to bring down all components to the bottom of the tubes. Don’t let the 96-well plate dry out since this will inactivate active components on the plate. Don’t reuse tips and tubes to avoid cross-contamination. Avoid using the reagents from different batches together. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

## KIT COMPONENTS/MATERIALS PROVIDED

|  |  |
| --- | --- |
| **Component** | **Quantity** |

## MATERIALS REQUIRED BUT NOT PROVIDED

• Microplate reader capable of reading absorbance at 450 nm. Incubator. • Automated plate washer (optional) • Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for a large numbers of samples.

## REAGENT PREPARATION

Bring all reagents to room temperature before use. Wash Buffer: Dilute Wash Buffer (25X) with distilled water. For example, if preparing 500 ml of Wash Buffer, dilute 20 ml of Wash Buffer (25X) into 480 ml of distilled water. Standard: Reconstitute the standard with standard diluent according to the label instructions. This reconstitution produces a stock solution. Let the standard stand for a minimum of 15 minutes with gentle agitation prior to making dilutions. Detection Reagent A and B: Dilute to the working concentration using Assay Diluent A and B, respectively.

## SAMPLE PREPARATION

## DILUTION OF STANDARD

1. Label 7 tubes, one for each standard: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml. 2. Pipette 300 µl of the Sample Diluent into each tube. 3. Pipette 300 µl of the reconstituted standard into the first tube and mix to create the 4000 pg/ml standard. 4. Pipette 300 µl from the 4000 pg/ml tube into the second tube and mix to create the 2000 pg/ml standard. 5. Continue this process for the remaining tubes. 6. The Sample Diluent serves as the zero standard (0 pg/ml).

## TYPICAL DATA / STANDARD CURVE

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

|  |  |
| --- | --- |
| **Concentration (pg/ml)** | **O.D.** |

[{'concentration': '0.0', 'od\_value': '0.119'}]

## INTRA/INTER-ASSAY VARIABILITY

- Intra-Assay Precision: Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision: Three samples of known concentration were tested in separate assays to assess inter-assay precision.

## ASSAY PROTOCOL

1. It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment, if you have missed this information). Prepare all reagents and working standards as directed previously. Remove excess microplate strips from the plate frame and seal and store them in the original packaging. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C). Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 100 µl of the prepared 1x Biotinylated Anti-Human SPINT1 antibody to each well. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C). Wash the plate 3 times with the 1x wash buffer: Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Repeat steps a-b 2 additional times. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C). Wash the plate 5 times with the 1x wash buffer: Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Repeat steps a-b 4 additional times. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.) Add 100 µl of Stop Solution to each well. The color should immediately change to yellow. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm. Assay Protocol Notes Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.

## DATA ANALYSIS

To analyze using manual methods, follow the process below: Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading. It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor. Background on SPINT1 Kunitz-type protease inhibitor 1 is an enzyme that in humans is encoded by the SPINT1 gene. It is a member of the Kunitz family of serine protease inhibitors. The SPINT1 gene encodes an inhibitor of hepatocyte growth factor activator (HGFAC) and matriptase and is involved in degradation of the extracellular matrix. SPINT1 is produced as an active membrane-associated protein and is proteolytically cleaved and secreted. Alternative splicing results in multiple variants encoding different isoforms. Human SPINT1/HAI-1 ELISA Kit

## DISCLAIMER

This material is sold for in-vitro use only in manufacturing and research. This material is not suitable for human use. It is the responsibility of the user to undertake sufficient verification and testing to determine the suitability of each product's application. The statements herein are offered for informational purposes only and are intended to be used solely for your consideration, investigation and verification.