# Rat TAP(Trypsinogen Activation Peptide) ELISA Kit

**Catalog #:KE1764**

**Detection and Quantification of Rat TAP(Trypsinogen Activation Peptide) in Serum, Plasma, Biological Fluids.**

**Please read the provided manual as suggested experimental protocols may have changed.**

**Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.**

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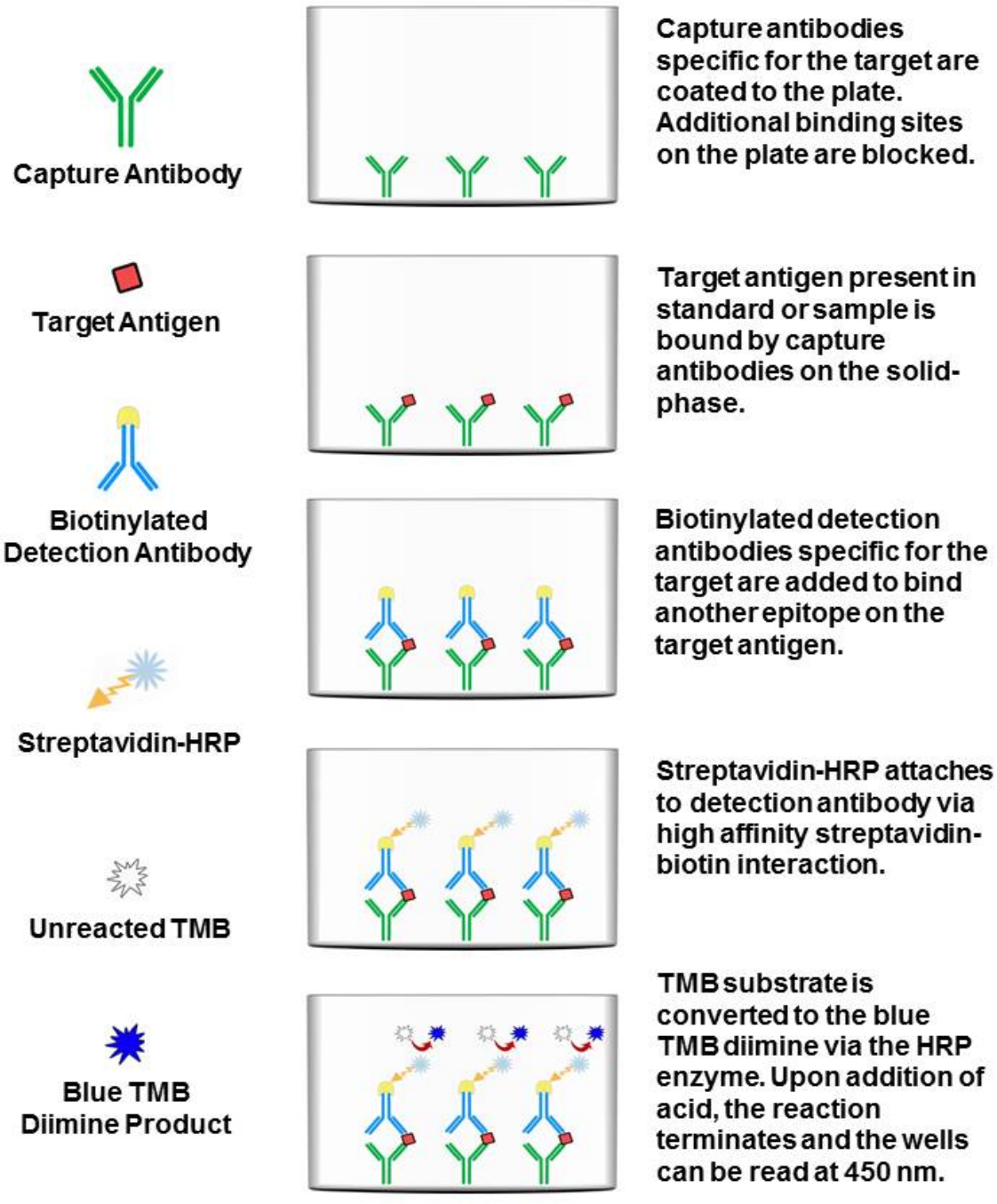
# ASSAY PRINCIPLES

The Rat TAP(Trypsinogen Activation Peptide) ELISA Kit contains the components necessary for quantitative determination of natural or recombinant Rat TAP(Trypsinogen Activation Peptide) concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a “Competive” Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a “Competive” with the primary capture antibodies in each well-bottom and the secondary detection antibodies added subsequently by the investigator.The capture antibodies coated to the bottom of each well are specific for a particular epitope on Rat TAP(Trypsinogen Activation Peptide) while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and “Competive” of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetricreaction to ensue upon substrate addition. When the substrate TMB (3, 3’, 5, 5’-Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.

# ASSAY FORMAT



# ASSAY RESTRICTIONS

- This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.

# MATERIALS INCLUDED

| Component | Quantity Per Plate |
|---|---|
| Microstrips Coated w/ Capture Antibody | 12 x 8-WellMicrostrips |
| Protein Standard | Lyophilized |
| Biotinylated Detection Antibody | 120µL |
| 100x Streptavidin-HRP | 120µL |
| Standard & Sample Diluent | 20 ml |
| Detection Antibody Diluent | 14 ml |
| Streptavidin-HRP Diluent | 14 ml |
| Ready-to-Use Substrate | 10 ml |
| Wash Buffer (25x) | 30 ml |
| Stop Solution | 10 ml |
| Adhesive Plate Sealers | 5 Sheets |
| Technical Manual | 1 Manual |

# ADDITIONAL MATERIALS REQUIRED

The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm)
- Micropipettes with capability of measuring volumes ranging from 1 µl to 1 ml
- Deionized or sterile water
- Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
- Graph paper or computer software capable of generating or displaying logarithmic functions
- Absorbent paper or vacuum aspirator
- Test tubes or microfuge tubes capable of storing ≥1 ml
- Bench-top centrifuge (optional)
- Bench-top vortex (optional)
- Orbital shaker (optional)

# HEALTH AND SAFETY PRECAUTIONS

- Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
- Stop Solution contains 2 N Sulfuric Acid ($H_2SO_4$) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

# STORAGE INFORMATION

**Note:** If used frequently, reagents may be stored at 4°C.

**Unopened Kits:** Store at 4°C for 6 months.

| Component | Storage Time | Storage Information |
|---|---|---|
| Microstrips Coated w/ Capture Antibody | 6 Months | 4°C |
| 100x Streptavidin-HRP | | |
| Wash Buffer (25x) | | |
| Assay Diluent | | |
| Ready-to-Use Substrate | | |
| Stop Solution | | |
| Biotinylated Detection Antibody | | |
| Protein Standard | Lyophilized: 6 Months<br>Reconstituted: 1 Month | |
| Adhesive Plate Sealers | - | - |
| Technical Manual | - | - |

# SAMPLE PREPARATION AND STORAGE

If samples are to be used within 24 hours, aliquot and store at 4°C. If samples are to be used over a long period of time, aliquot and store between -20°C and -80°C, depending on the duration of storage.

**Note:** Samples containing a visible precipitate or pellet must be clarified prior to use in the assay.

**Caution:**Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples.

### *Cell Lysate and Supernatants*

Remove large cell components via centrifugation and perform the assay. Cell lysates and supernatants require a dilution using Assay Diluent. A serial dilution may be performed to determine a suitable dilution factor for the sample. For future use of the sample, follow the sample storage guidelines stated above.

### *Serum*

Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. Serum samples may require a dilution using Assay Diluent. For future use of the sample, follow the storage guidelines above.

### *Plasma*

Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. Plasma samples may require a dilution using Assay Diluent. Afterwards, perform the assay or for future use of the sample, follow the storage guidelines stated above.

# SAMPLE EXPERIMENT LAYOUT

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **A** | Standard (High Point) | Standard (High Point) | Standard (High Point) | Sample | Sample | Sample |
| **B** | Standard (1:2) | Standard (1:2) | Standard (1:2) | Sample | Sample | Sample |
| **C** | Standard (1:4) | Standard (1:4) | Standard (1:4) | Sample | Sample | Sample |
| **D** | Standard (1:8) | Standard (1:8) | Standard (1:8) | Sample | Sample | Sample |
| **E** | Standard (1:16) | Standard (1:16) | Standard (1:16) | Sample | Sample | Sample |
| **F** | Standard (1:32) | Standard (1:32) | Standard (1:32) | Sample | Sample | Sample |
| **G** | Standard (1:64) | Standard (1:64) | Standard (1:64) | Sample | Sample | Sample |
| **H** | Negative Control | Negative Control | Negative Control | Sample | Sample | Sample |

# IMMUNOASSAY PROTOCOL

**Note:**If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use.

**Note:**Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom.

### *Reconstitution of Provided Materials*

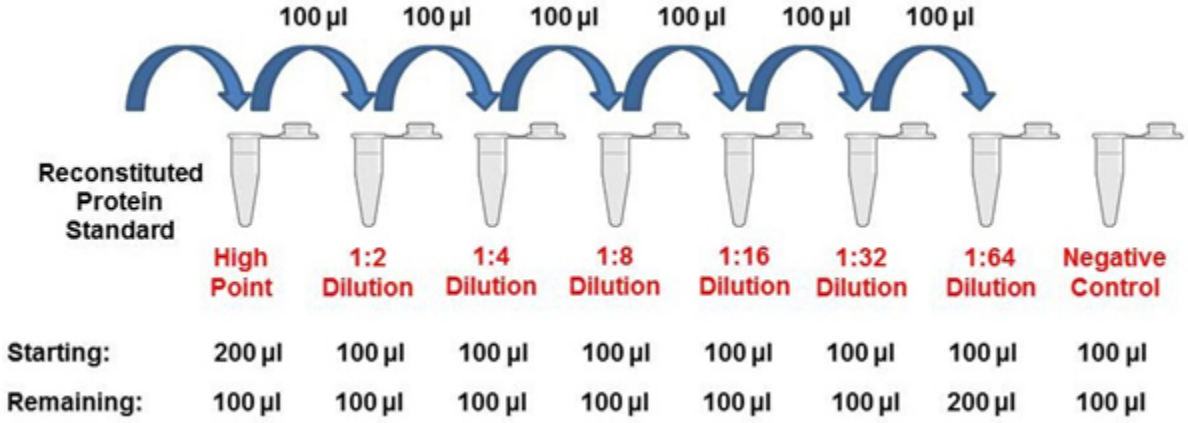
1. Reconstitute the Biotin-Conjugated Detection Antibody in Detection Antibody Diluent(1:100)
2. Reconstitute the Protein Standard in 1mL Standard & Sample Diluent for 10ng/mL.
3. Dilute the 25x Wash Buffer in dd$H_2O$ for 750 ml of 1x Wash Buffer.
4. Reconstitute the 100x Streptavidin-HRP in 100x Streptavidin-HRP Diluent(1:100)

### *Addition of Known Standard and Unknown Sample to Immunoassay*

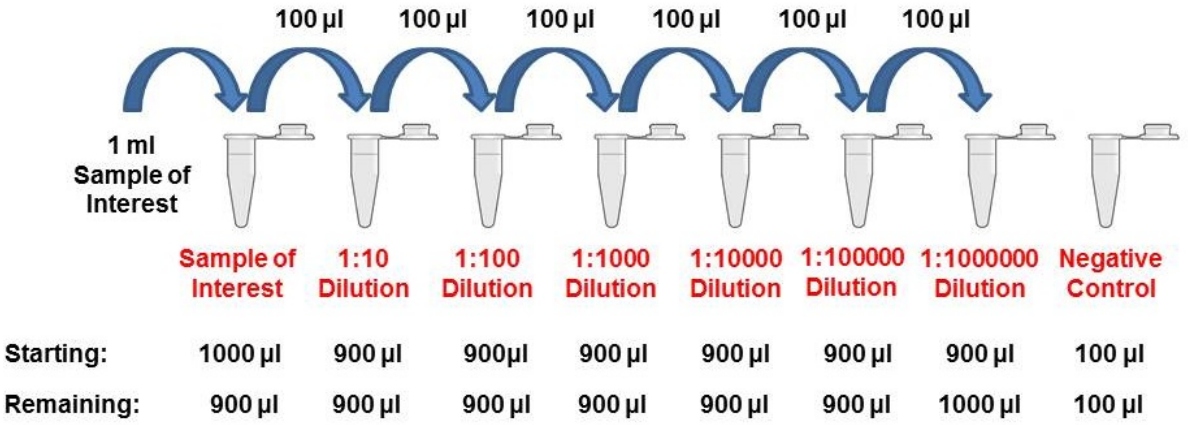
The Rat TAP(Trypsinogen Activation Peptide) ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Rat TAP(Trypsinogen Activation Peptide) within the range of 0-10ng/mL.

1. Dilute the known standard from 10ng/mL to 0 in a series of microfuge tubes using Standard & Sample Diluent. Mix each tube thoroughly by inverting several times or by vortexing lightly to ensure proper equilibration. Add 50 µl of each serial dilution step into the wells of a specified row or column of the 96-well microtiter plate in duplicate or triplicate. Unknown samples of interest can be serial diluted withAssay Diluent to concentrations within the detection range of this assay kit and added to the plate at 50 µl per well.

To obtain serial dilution high point, dilute reconstituted Protein Standard to the maximum concentration for serial dilution by adding *1000*µl reconstituted Protein Standard to serial dilution high point tube and then raising the volume to 200 µl. Shown below is a diagram illustrating a hypothetical 2-fold serial dilution on a given reconstituted Protein Standard.




For samples of unknown protein concentrations, serial dilute the experimental sample using Standard & Sample Diluent to determine range of detection and acceptable dilutions. Shown below is a diagram illustrating a 10-fold serial dilution on a given Sample of Interest.



### *Addition of Detection Antibody to Capture Antibody-Bound Samples*

1. Dilute the detection antibody solution 1:100 in detection antibody Diluent. Mix the test tube either by inverting several times or vortexing to ensure proper equilibration. Add 50 µl of the diluted detection antibody solution into each well, Seal the microplate air-tight using one of the microplate adhesive seals provided in this kit or Parafilm if readily available.
2. Seal the plate and incubate at 37° C for 45min.

### *Conjugation of Streptavidin-HRP to Biotinylated Detection Antibody*

1. Remove the detection solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Perform 4 consecutive wash steps with gentle shaking between each wash, using 350µl washing buffer per well, 1-2minutes for each wash.

2. Dilute the 100x Streptavidin-HRP by 1:100 using Streptavidin-HRP Diluent to a 1x Streptavidin-HRP solution.

3. After the 4$^{th}$ wash step, add 100 µl of 1xStreptavidin-HRP solution into each well and incubate at room temperature for 60 minutes.

### *Application of Liquid Substrate for Colorimetric Reaction*

1. Remove the 1xStreptavidin-HRP solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Prepare the Ready-to-Use Substrate by bringing it to room temperature without exposure to fluorescent or UV light as these may degrade the substrate. Perform 4 consecutive wash steps with gentle shaking between each wash.

2. After the 4$^{th}$ wash step, add 100 µl of Ready-to-Use Substrate solution into each well and incubate at room temperature for color development. The microplate should be kept out of direct light by either covering with an opaque object or putting it into a dark room. Closely monitor the color development as some wells may turn blue

very quickly depending on analyte and/or detection antibody-HRP concentrations. Once the blue color has ceased to develop further, immediately add 50 µl of Stop Solution to each well being used. The color in the wells should immediately change from blue to yellow.

3. The microplate is now ready to be read by a microplate reader. Within 30 minutes of adding the Stop Solution, determine the optical density (absorbance) of each well by reading the plate with the microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. **Caution:** Readings made directly at 450 nm without correction may be higher and less accurate.

***Generation of Standard Curve and Interpretation of Data***

1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.

2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). **Note:** Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or “trend-line” through the plotted points via regression analysis. **Note:** Shown on the next page is an example of typical data produced by analysis of the standard sample.

## SUMMARIZED PROTOCOL

Reconstitute the Biotin-Antibody, Protein Standard, 25x Wash Buffer. HRP-antibody as specified

Add 50 µl detection antibody and 50 µl samples(Standard ) same time, incubate at 37°C for 45min.

Add 350µl washing buffer per well, 1-2minutes for 4 steps each wash.

Add 100 µl of 1xStreptavidin-HRP solution and incubate at room temperature for 60 minutes.

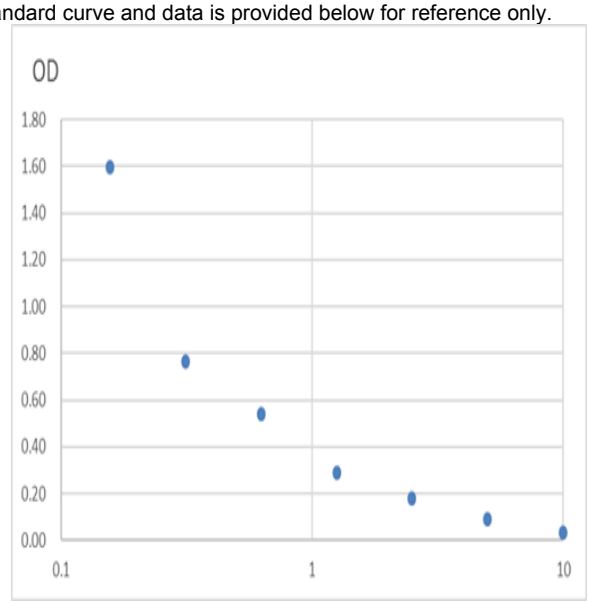
4 steps wash

Add 100 µl of Ready-to-Use Substrate solution and incubate at room temperature for 15min

Add 50 µl of Stop Solution and read plate at 450nm

# TYPICAL DATA

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.



# SENSITIVITY

The Rat TAP(Trypsinogen Activation Peptide) ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Rat TAP(Trypsinogen Activation Peptide) within the range of 0-10ng/mL and Sensitivity: 0.094ng/mL.

# TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com.

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## NOTES


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