KAMIYA BIOMEDICAL COMPANY

# Human TRAP-5b EIA

**For the quantitative determination of tartrate-resistant acid phosphatase isoform 5b in human serum**

Cat. No. KT-652

**For Research Use Only.**

## PRODUCT INFORMATION

# Human TRAP-5b EIA

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## INTENDED USE

The **K-*ASSAY*®** Human TRAP-5b EIA is an immunocapture enzyme assay for the determination of osteoclast-derived tartrate-resistant acid phosphatase isoform 5b (TRAP-5b) in human serum. For research use only.

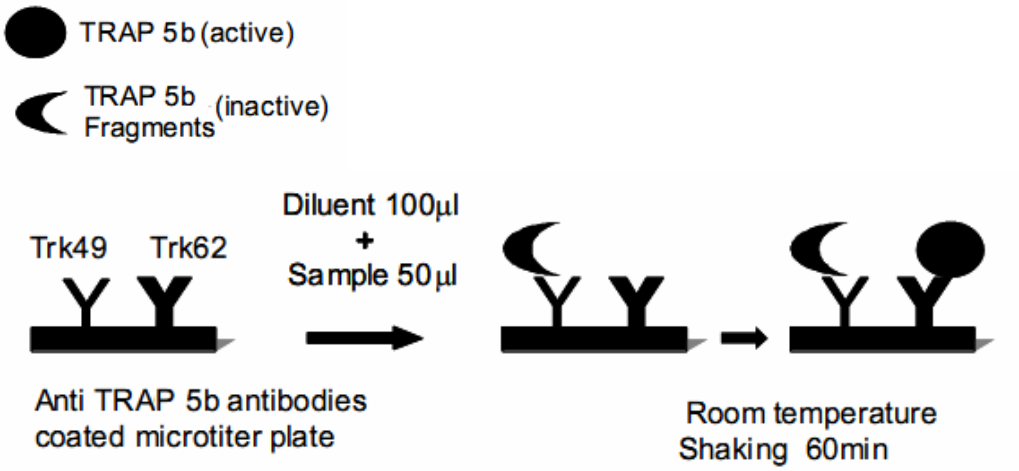
## INTRODUCTION

TRAP-5b is typically expressed in osteoclasts and then secreted into blood circulation. Therefore, serum TRAP-5b has been regarded as a useful marker for bone resorption. The **K-*ASSAY*®** Human TRAP-5b EIA features high specificity for the 5b isoform of tartrate-resistant acid phosphatase in serum. By utilizing specific monoclonal antibodies to capture the 5b isoform, it avoids interference from TRAP-5a, which is not specific for bone resorbing osteoclasts. Research indicates that TRAP-5b would be useful as a marker of bone resorption and osteoclast activity in research studies. The **K-*ASSAY*®** Human TRAP-5b EIA is a specific assay, with good precision and a wide range of linearity.

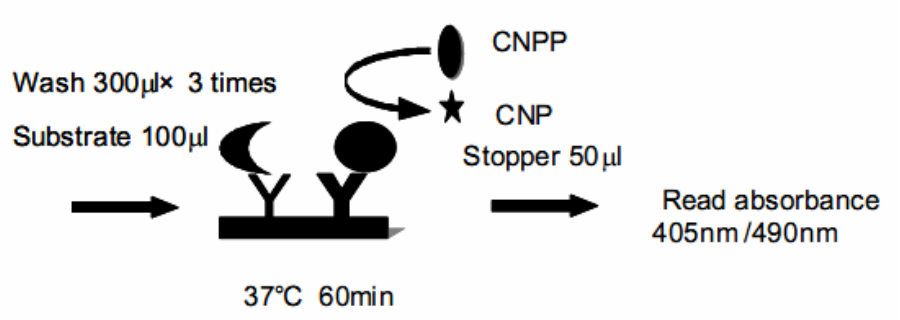
## PRINCIPLE

**The K-*ASSAY*®** Human TRAP-5b EIA detects the enzyme activity of TRAP-5b based on a novel assay system called fragments absorbed immunocapture enzymatic assay (FAICEA). The assay uses two unique monoclonal antibodies. The first antibody, Trk49 has high specificity with inactive TRAP-5b fragments and the second antibody, Trk62 has a high specificity for intact TRAP-5b. Therefore, Trk49 reacts with inactive TRAP-5b fragments and it enables Trk62 to easily react with active TRAP-5b in the reaction mixture. After immunoreaction, TRAP-5b activity is measured by 2-chloro-4-nitrophenyl phosphate (CNPP) as a substrate. The activities are calculated by using a 5 point calibration curve.

1) Pipette 100 µL of diluent and 50 µL of sample.
2) Incubation (Room temperature), Shaking, 60 min.




4) Wash (300 µL x 3 times).
5) Pipette 100 µL of substrate solution.
6) Incubation (37℃, 60 min).




7) Pipette 50 µL of Stop solution.
8) Read the absorbance (405 / 490 nm).

## COMPONENTS

1. Microplate
   12 x 8 wells coated with monoclonal anti-TRAP-5b antibodies (mouse).

2. Sample Diluent
   1 bottle of 20 mL Tris buffer.

3. Calibrator A-E
   2 vials with 0.4 mL each concentration, Human TRAP-5b, lyophilized. The exact concentration is stated on each vial.

4. Control I and II
   2 vials with 0.4 mL each concentration, Human TRAP-5b, lyophilized. The exact concentration is stated on each vial.

5. Substrate
   2 vials with 12 mL of substrate dissolving solution each, 2-Chloro-4-nitrophenyl phosphate, powder.

6. Substrate Dissolving Solution
   2 bottles of 12 mL MES buffer.

7. Stop Solution
   1 bottle of 12 mL 0.2M sodium hydroxide.

8. Wash Buffer Concentrate (10X)
   1 bottle of 100 mL TBS/Tween.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Adjustable micropipettes for dispensing 50, 100, 300 µL, single and multi-channel.
- Glass or plastic containers for dilution of wash buffer.
- Microplate shaker capable of constant shaking at 500-1000 rpm for 60 min.
- Distilled or de-ionized water.
- Incubator capable of maintaining 37±1℃.
- Suitable device for washing of microplate.
- Microplate spectrophotometer.

## REAGENT PREPARATION

- Bring all reagents to room temperature (20-25℃) before use.
- Thoroughly mix the reagents before use by gently agitating or swirling.
- To prepare the Wash solution, dilute one volume of Wash Buffer Concentrate into 9 volumes of distilled water.
- To prepare the Substrate reagent, dissolve in 12 mL of Substrate Dissolving Solution.
- To prepare the Calibrators and Controls, reconstitute each vial with 0.4 mL of distilled water.

## PLATE CONFIGURATION

An example is below:

| | 1 | 2 | 3 |
|---|---|---|---|
| A | Calibrator A | Calibrator E | Sample #2 |
| B | Calibrator A | Calibrator E | Sample #2 |
| C | Calibrator B | Control 1 | Sample #3 |
| D | Calibrator B | Control 1 | Sample #3 |
| E | Calibrator C | Control 2 | Sample #4 |
| F | Calibrator C | Control 2 | Sample #4 |
| G | Calibrator D | Sample #1 | Sample #5 |
| H | Calibrator D | Sample #1 | Sample #5 |

## STORAGE

Store the kit at 4℃ until the expiration date.

## SAMPLE COLLECTION AND HANDLING

Collect serum using standard venipuncture technique. Samples should be collected without anticoagulants and in a manner that prevents hemolysis. Allow blood to clot and separate the serum by centrifugation. Serum can be stored for 2 days at 4℃, for one month if stored between -20 and -30℃, or for 24 months at -80℃. Do not subject samples to freeze/thaw cycles.

## ASSAY PROCEDURE

1. Pipette 100 µL of sample diluent into wells.

2. Pipette 50 µL of Calibrators, Controls and samples into their respective wells.

3. Seal the microplate with included microplate sealer and incubate for 60 min at room temperature on a microplate shaker set at 500-1000 rpm.

4. After incubation, wash the microplate wells three times with 300 µL of wash buffer per well. After washing tap wells on paper towels.

5. Pipette 100 µL of Substrate solution into each well.

6. Seal the microplate with the microplate sealer and shake it on a microplate shaker at room temperature for 30 sec at 500-1000 rpm. Then, incubate for 60 min in an incubator at 37℃ without shaking.

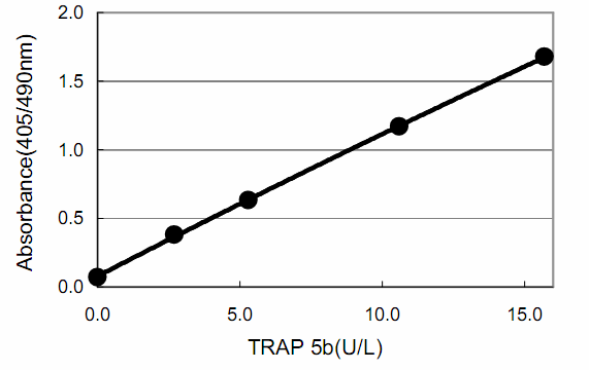
7. Pipette 50 µL of Stop solution into each of the wells.

8. Read and record the absorbance of each well at 405 nm with a 490 nm reference filter.

9. Calculate the value of the Controls and samples from the calibration curve.

## CALIBRATION CURVE

The following is a representative calibration curve. Do not use this calibration curve for your experiment. It is only provided to represent a typical calibration curve.




## REFERENCE VALUES

Reference values for TRAP-5b activity in healthy Japanese men and women were defined as follows.

*Reference intervals = Logarithmic mean ± 1.96 SD.

| Group | Age | Number of Subjects | Reference Interval*(U/L) |
|---|---|---|---|
| Men | 20-82 | 319 | 1.7 – 5.9 |
| Premenopausal women | 33-44 | 188 | 1.2 – 4.2 |

## PERFORMANCE CHARACTERISTICS

### Sensitivity

The minimum detection limit of the **K-ASSAY®** Human TRAP-5b EIA is 0.1 U/L, determined by the upper 3 SD limit in a zero standard precision study.

### Recovery

Spike recovery was determined by adding a known quantity of purified TRAP-5b to serum samples with different levels of endogenous TRAP-5b. Typical results are provided after spiking serum samples with low and high concentrations of TRAP-5b and assaying in duplicate.

Spike Recovery: 92-103%

### Precision

Intra-assay precision was determined for 16 replicates of 2 serum samples. Inter-assay was determined for 8 replicates over 7 days with 2 serum samples.

| | TRAP-5b (U/L) | SD (U/L) | CV% |
|---|---|---|---|
| **Intra-assay** | 3.4 | 0.07 | 2.2 |
| | 7.4 | 0.14 | 1.9 |
| **Inter-assay** | 3.8 | 0.11 | 3.0 |
| | 7.4 | 0.15 | 2.0 |

### Assay Range

0.1 – 15 U/L

## PRECAUTIONS

1) Human serum should be handled in a careful and safe manner.
2) Please avoid cross-contamination between the reagents.

3) Do not mix reagents from different lots, as this can have a negative effect on the performance and precision of the product.
4) Stop solution is 0.2M NaOH, please handle in a careful and safe manner.
5) Prepare the substrate reagent just before use.
6) A separate calibration curve must be created for each assay run.
7) Calibrators and controls have been tested and found negative for antibodies to HIV, HCV, and HBsAg, however no test can offer complete assurance that HIV, HCV, and HBsAg are absent. Therefore they should be handled in the same manner as potentially infectious material.

**FOR RESEARCH USE ONLY**

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