ACP5 (Human) ELISA Kit

Catalog Number KA0041

96 assays

Version: 08

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

The ACP5 (Human) ELISA Kit is an ImmunoCapture Enzyme-Activity Assay for the quantitative measurement of human TRAP 5 in serum and EDTA plasma.

- Features
- ✓ For research use only.
- ✓ The total assay time is less than 4 hours.
- ✓ The kit measures TRAP 5 proteins exhibiting enzyme activity in serum and EDTA plasma
- ✓ Assay format is 96 wells.
- ✓ Quality Controls are recombinant TRAP 5 protein based. No animal sera are used.
- ✓ Calibrators are recombinant protein based.
- ✓ Components of the kit are provided ready to use, concentrated or lyophilized

Background

TRAP 5 (serum band 5 tartrate-resistant acid phosphatase, TRACP 5; EC 3.1.3.2) is a glycoprotein of 35-37 kDa. TRAP 5 belongs to the most abundant enzymes in osteoclasts. It is expressed in certain differentiated cells of the mononuclear phagocyte system, particularly osteoclasts and alveolar macrophages, where it takes an active part in bone resorption process.
High blood levels of TRAP 5 are usually associated with active bone remodeling. Increased serum levels are observed during normal bone growth among healthy children.
Elevated serum TRAP levels have been detected in diseases characterized by increased bone resorption; Paget's disease of the bone, hemodialysis, primary hyperparathyroidism, metastatic malignancies involving bone resorption, multiple myeloma and bilaterally ovariectomized women. Post-menopausal women have higher levels of serum than post-menopausal women on estrogen replacement therapy. Therefore specific determination of TRAP 5 activity can be essential for clinical assessment of bone metabolism

Principle of the Assay

In the ACP5 (Human) ELISA Kit, calibrators, Quality Control and samples are incubated in microplate wells pre-coated with monoclonal anti-human TRAP 5 antibody. After a thorough wash, TRAP 5 bound to the antibody is allowed to react with the pNPP substrate at pH 5.5. The reaction is stopped by addition of hydroxide solution and absorbance of the resulting yellow colour product is measured. The absorbance is proportional to the enzymatic activity of TRAP 5. A standard curve is constructed by plotting absorbance values against enzyme activities of recombinant TRAP 5 calibrators, and enzyme activity of unknown samples are determined (U/L) using this calibration curve.

General Information

Materials Supplied

List of component

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Dissociating solution	ready to use	7 mL
Set of Calibrator (0.2-4 U/L)	lyophilized	2 sets
Quality Control	lyophilized	2 vials
Dilution Buffer	ready to use	20 mL
Wash Solution Conc. (10x)	concentrated	100 mL
Substrate Buffer	-	20 mL x 2
Substrate Tablets	-	4 psc
Stop Solution	ready to use	13 mL

Storage Instruction

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).
For stability of opened reagents see Reagent Preparation section.

Materials Required but Not Supplied

- ✓ Deionized (distilled) water
- ✓ Test tubes for diluting samples
- ✓ Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- ✓ Precision pipettes to deliver 50-200 µL with disposable tips
- ✓ Multichannel pipette to deliver 50-200 µL with disposable tips
- ✓ Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- ✓ Vortex mixer
- ✓ Orbital microplate shaker capable of approximately 300 rpm
- ✓ Microplate washer (optional). [Manual washing is possible but not preferable.]
- ✓ Microplate reader with 405 ± 10 nm filter, preferably with reference wavelength 620 nm - 650 nm
- ✓ Software package facilitating data generation and analysis (optional)

Precautions for Use

- Precautions

✓ For research use only.

✓ Wear gloves and laboratory coats when handling immunodiagnostic materials.

✓ Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

✓ Avoid contact with the corrosive alkaline Stop Solution and Substrate Solution, which contains pNPP. Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.

✓ The materials must not be pipetted by mouth.

- Technical hints

✓ Reagents with different lot numbers should not be mixed.

✓ Use thoroughly clean glassware.

✓ Use deionized (distilled) water, stored in clean containers.

✓ Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.

✓ Substrate Solution is colourless during incubation until its pH is changed by adding the Stop Solution.

✓ Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from colourless to yellow immediately after the addition of the Stop Solution.

✓ Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

Assay Protocol

Reagent Preparation

- ✓ All reagents need to be brought to room temperature prior to use.
- ✓ Always prepare only the appropriate quantity of reagents for your test.
- ✓ Do not use components after the expiration date marked on their label.

- Assay reagents supplied ready to use:

✓ Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

✓ Dissociating Solution

- Dilution Buffer
- Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

✓ Human TRAP 5 Calibrators

Reconstitute the lyophilized Calibrators just prior to the assay. Add 270 µL of deionized (distilled) water to the vial containing lyophilized Calibrator 4, 260 µL of deionized (distilled) water to the vial containing lyophilized Calibrator 2 and 260 µL of deionized (distilled) water to the vial containing lyophilized Calibrator 0.2. Let it dissolve at least 15 minutes and mix thoroughly.

Prepared Calibrators are ready to use, do not dilute them.

Stability and storage:

The reconstituted Calibrators have to be used immediately or to be stored frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

✓ Quality Control

Refer to the Certificate of Analysis for current Quality Control concentration!!!

Reconstitute Quality Control (QC) just prior to the assay. Add 60 µL of deionized (distilled) water to the vial containing lyophilized Quality Control. Let it dissolve at least 15 minutes and mix thoroughly.

Dilute reconstituted Quality Control 4x with Dilution Buffer, e.g. 60 µL of Quality Control + 180 µL of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Note: Concentration of analyte in Quality Control need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Control serves just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Stability and storage:

The reconstituted Quality Control must be used immediately or to be stored frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Control.

- ✓ Substrate Solution

  Add 1 Substrate Tablet to 10 mL of the Substrate Buffer, let it dissolve and mix the solution thoroughly. The solution should be prepared before use, 10-15 minutes is needed to dissolve the tablet.

  Stability and storage:

  The Substrate Solution must be used immediately.

- ✓ Wash Solution Conc. (10x)

  Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 mL of Wash Solution Concentrate (10x) + 900 mL of distilled water for use of all 96-wells.

  Stability and storage:

  The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

Sample Preparation

The kit measures TRAP 5 in serum or EDTA plasma.

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 4x with Dilution Buffer just prior to the assay, e.g. 60 µL of sample + 180 µL of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored for 1 day at + 4°C, for 1 month at -20°C, or preferably at -70°C for six months. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

Assay Procedure

1. Pipet 100 µL of each Calibrators, diluted Quality Control, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See Plate Layout.
2. Pipet 50 µL of Dissociating Solution into all the used wells.
3. Incubate the plate at room temperature (ca. 25°C) for 2 hours, shaking at ca. 300 rpm on an orbital microplate shaker.
4. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
5. Add 100 µL of Substrate Solution into each well.
6. Incubate the plate in an incubator at 37°C for 1.5 hour, no shaking!
7. Stop the enzyme reaction by adding 100 µL of Stop Solution.
8. Determine the absorbance of each well using a microplate reader set to 405 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 620 -650 nm). Subtract readings at 630 nm (620-650 nm) from the readings at 405 nm. The absorbance should be read within 5 minutes following step 7.

Warning

THE SUBSTRATE SOLUTION IS COLOURLESS DURING INCUBATION UNTIL ITS pH IS CHANGED BY ADDING THE STOP SOLUTION!!!

Note: Manual washing: Aspirate wells and pipet 0.35 mL Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

Data Analysis

Calculation of Results

Most microplate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the mean absorbance (Y) of Calibrators against the known enzyme activity (X) of Calibrators, using linear regression function. Results are reported as enzyme activity of TRAP 5 (U/L) in samples.

The measured enzyme activity of samples and Quality Control calculated from the calibration curve must be multiplied by their respective dilution factor, because samples and Quality Control have been diluted prior to the assay; e.g. 2.5 U/L (from calibration curve) x 4 (dilution factor) = 10 U/L.

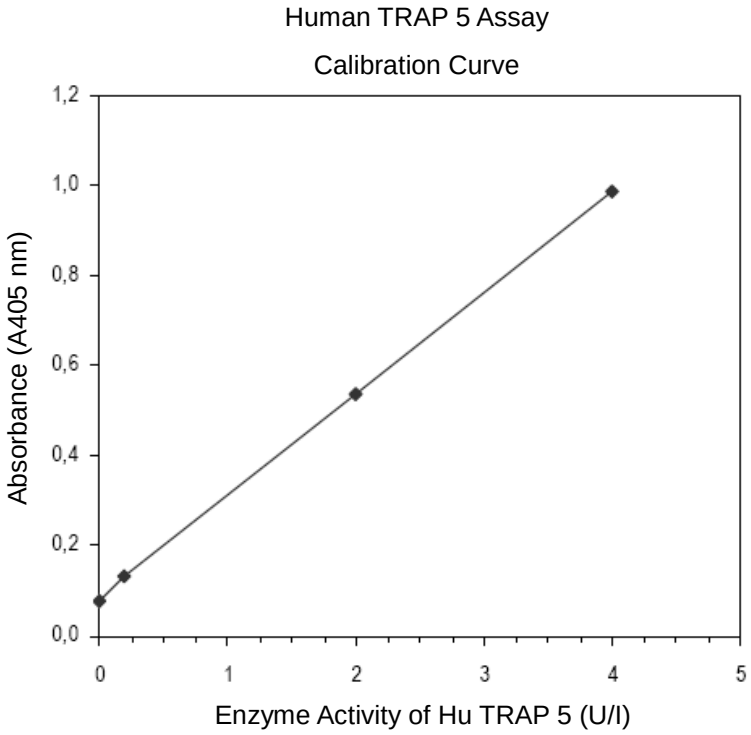

Figure 1: Typical Standard Curve for ACP5 (Human) ELISA Kit.

Performance Characteristics

- Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3xSD_{blank}) is calculated from the real TRAP 5 values in wells and is 0.01 U/L. *Note: Dilution Buffer is pipetted into blank wells.*

- Limit of assay

Results exceeding TRAP 5 level of 16 U/L should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the TRAP 5 concentration.

- Specificity

The antibodies used in this Assay are specific for human TRAP 5. Determination of TRAP 5 does not interfere with hemoglobin (1.0 mg/mL), bilirubin (170 umol/L) and triglycerides (5.0 mmol/L).

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	yes
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	yes
Rat	no
Sheep	no

- Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (U/L)	Standard Deviation (U/L)	CV (%)
1	1.88	0.00	0.8
2	2.20	0.01	2.4

Inter-assay (Run-to-Run) (n=8)

Sample	Mean (U/L)	Standard Deviation (U/L)	CV (%)
1	2.10	0.13	6.4
2	2.97	0.23	7.6

- Spiking Recovery

Serum samples were spiked with different amounts of human TRAP 5 and assayed.

Sample	**O**bserved (U/L)	**E**xpected (U/L)	Recovery **O/E** (%)
1	2.67	-	-
	2.80	2.87	98
	4.47	4.67	96
	6.26	6.67	94
2	3.26	-	-
	3.17	3.46	92
	4.81	5.26	91
	6.76	7.26	93

- Linearity

Serum samples were serially diluted with Dilution Buffer (4x) and assayed.

Sample	Dilution	**O**bserved (U/L)	**E**xpected (U/L)	Recovery **O/E** (%)
1	-	7.83	-	-
	2x	4.31	3.92	110
	4x	2.05	1.96	105
	8x	1.05	0.98	107
2	-	5.27	-	-
	2x	2.63	2.64	100
	4x	1.37	1.32	104
	8x	0.71	0.66	108

- Reference Ranges

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and abnormal references ranges for TRAP 5 levels with the assay.

a) The following results were obtained when 177 random samples were analysed with ACP5 (Human) ELISA.

Gender	Age (years)	n	Mean (U/l)	SD (U/l)	Min (U/l)	Max (U/l)
Men	8-14	5	16.18	3.29	12.25	20.38
	18-48	16	4.54	0.58	3.70	5.61
	51-84	30	4.47	1.90	1.56	12.50
Women	4-14	8	15.58	4.28	9.40	22.26
	16-50	63	3.79	0.85	2.12	6.32
	52-90	55	4.68	1.49	2.38	11.69

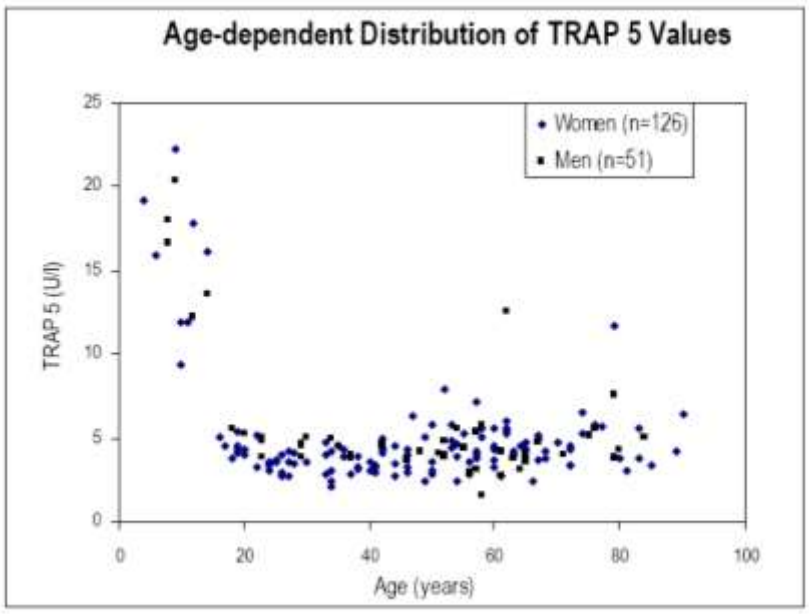

Figure 2: Age-dependent distribution of TRAP 5 values.

b) TRAP 5 levels were determined in 489 individuals with osteopathy and were compared with those of osteocalcin and DPD/Kr.

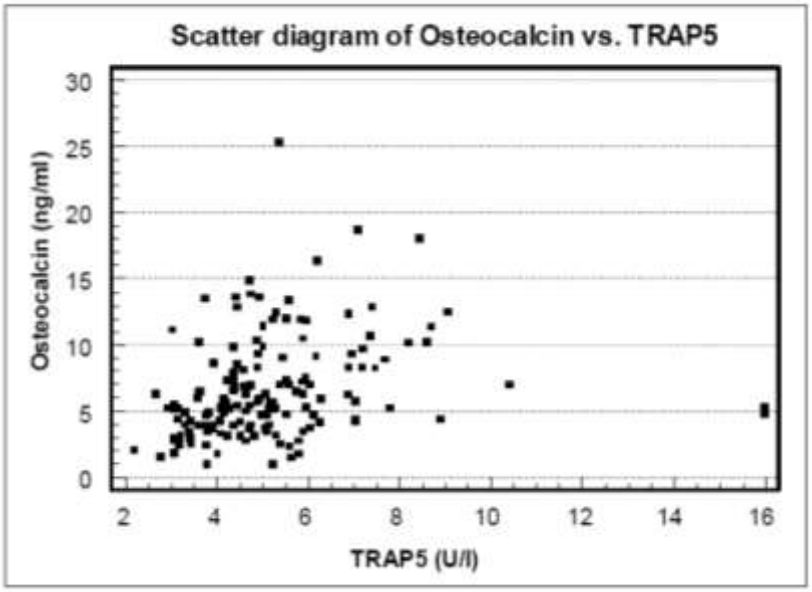

Figure 3: Scatter diagram of osteocalcin vs. TRAP 5.

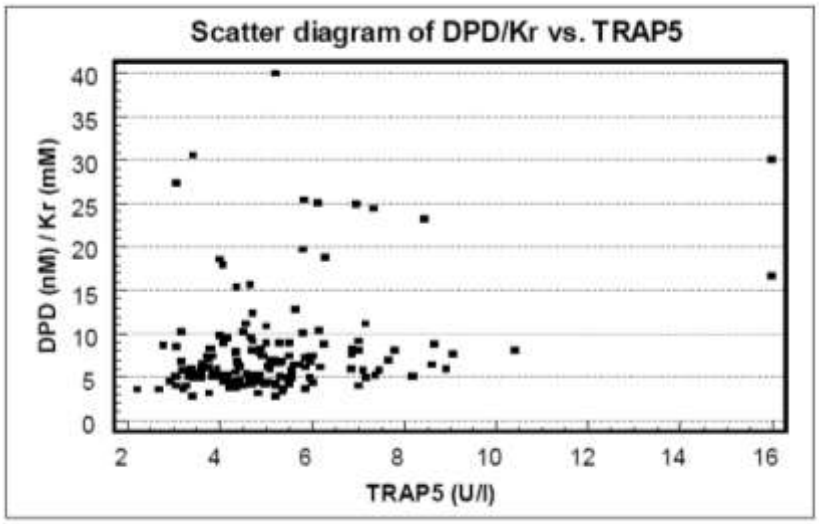

Figure 4: Scatter diagram of DPD/Kr vs. TRAP 5.

c) Diurnal variation of TRAP 5 levels in serum was determined in 8 individuals in the course of 12 hours.

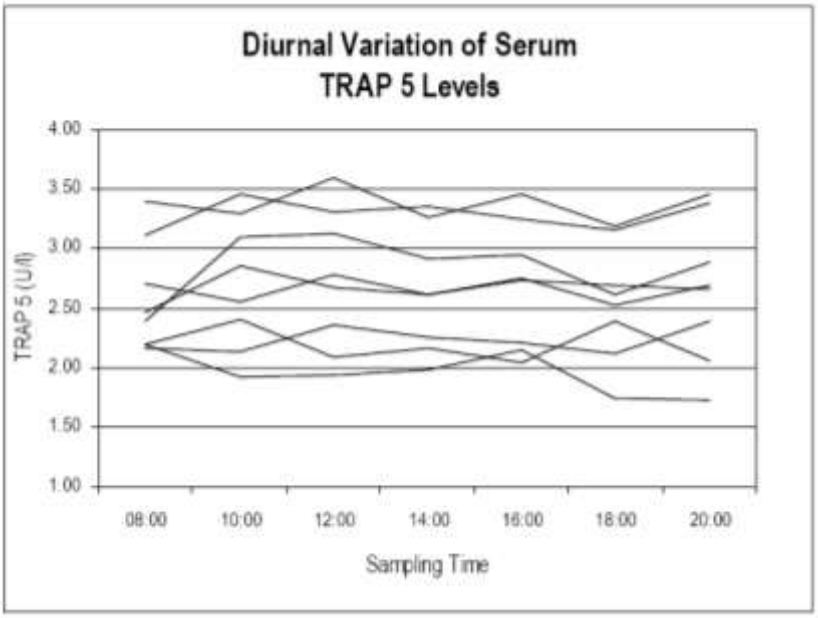

Figure 5: Diurnal variation of serum TRAP 5 levels.

- Definition of the Calibrators

The recombinant protein is used as the Calibrator in this assay. Recombinant human TRAP is expressed in Sf 9 cell.

- Method Comparison

The ACP5 (Human) ELISA Kit was compared to a commercial assay specific for TRAP 5b isoform. Linear regression analysis of the results yielded the following results:

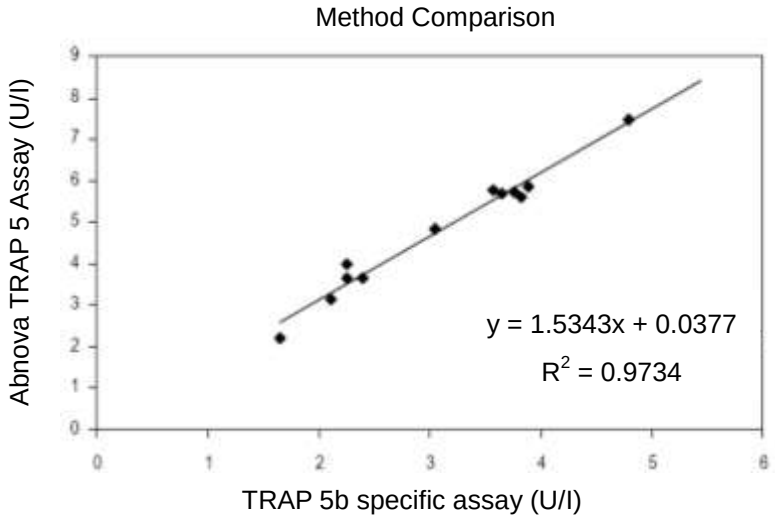

Figure 6: Method comparison.

Resources

Troubleshooting

Weak signal in all wells

Possible explanations:

- ✓ Omission of a reagent or a step
- ✓ Improper preparation or storage of a reagent
- ✓ Assay performed before reagents were allowed to come to room temperature
- ✓ Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- ✓ Improper or inadequate washing
- ✓ Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- ✓ Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- ✓ Improper or inadequate washing
- ✓ Improper mixing Calibrators, Quality Controls or samples

References

1. Minkin C. Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif Tissue Int. 1982, 34, 285-290.
2. Lau KH, Onishi T, Wergedal JE, Singer FR, Baylink DJ. Characterization and assay of tartrate-resistant acid phosphatase activity in serum: potential use to assess bone resorption. Clin Chem. 1987, 33, 458-462.
3. Nakanishi M, Yoh K, Uchida K, Maruo S, Matsuoka A. Improved method for measuring tartrate-resistant acid phosphatase activity in serum. Clin Chem. 1998, 44, 221-225.
4. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Vaananen HK. Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. J Bone Miner Res. 2000, 15, 133-1345.
5. Lamp EC, Drexler HG. Biology of tartrate-resistant acid phosphatase. Leuk Lymphoma. 2000, 39, 477-484.
6. Nakanishi M, Yoh K, Miura T, Ohasi T, Rai SK, Uchida K. Development of a kinetic assay for band 5b tartrate-resistant acid phosphatase activity in serum. Clin Chem. 2000, 46, 469-473.

7. Waguespack SG, Hui SL, White KE, Buckwalter KA, Econs MJ. Measurement of tartrate-resistant acid phosphatase and the brain isoenzyme of creatine kinase accurately diagnose type II autosomal dominant osteopetrosis but does not identify gene carriers. J Clin Endocrinol Metab. 2002, 87, 2212-2217.
8. Igarashi Y, Lee M, Matsuzaki S. Acid phosphatases as markers of bone metabolism. J Chromatogr B. 2002, 781, 345-358.
9. Terpos E, de la Fuente J, Szydlo R, Hatjiharissi E, Viniou N, Meletis J, Yataganas X, Goldman JM, Rahemtulla A. Tartrate-resistant acid phosphatase isoform 5b: a novel serum markerfor monitoring bone disease in multiple myeloma. Int J Cancer. 2003, 106, 455-457.
10. Monier-Faugere MC, Mawad H, Malluche HH. Opposite effects of calcitriol and paricalcitol on the parathyroid hormone-(1-84)/large carboxy-terminal-parathyroid hormone fragments ratio in patients with stage 5 chronic kidney disease. Clin J Am Soc Nephrol. 2007, 6, 1255-60.
11. Halleen JM, Tiitinen SL, Ylipahkala H, Fagerlund KM, Vaananen HK. Tartratr-resistant Acid Phosphatase 5b (TRAP 5b) as a Marker of Bone Resorption. Clin Lab. 2006, 52, 499-509.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Calibrator 4	Calibrator 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
B	Calibrator 2	Calibrator 2	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
C	Calibrator 0.2	Calibrator 0.2	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
D	Blank	Blank	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
E	Quality Control	Quality Control	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40
F	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33	Sample 41	Sample 41
G	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34	Sample 42	Sample 42
H	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35	Sample 43	Sample 43