

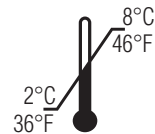
# MICROVUE™ TRAP5b EIA Kit

Bone Health

An immunocapture enzyme assay for the determination of tartrate-resistant acid phosphatase isoform 5b in human serum or plasma

CE Version — FOR EXPORT ONLY  
This Version Not for Sale or Distribution in the U.S.

English



**QUIDEL**®  
CORPORATION  
SPECIALTY PRODUCTS  
RESEARCH TO RAPIDS®

**REF** 8036

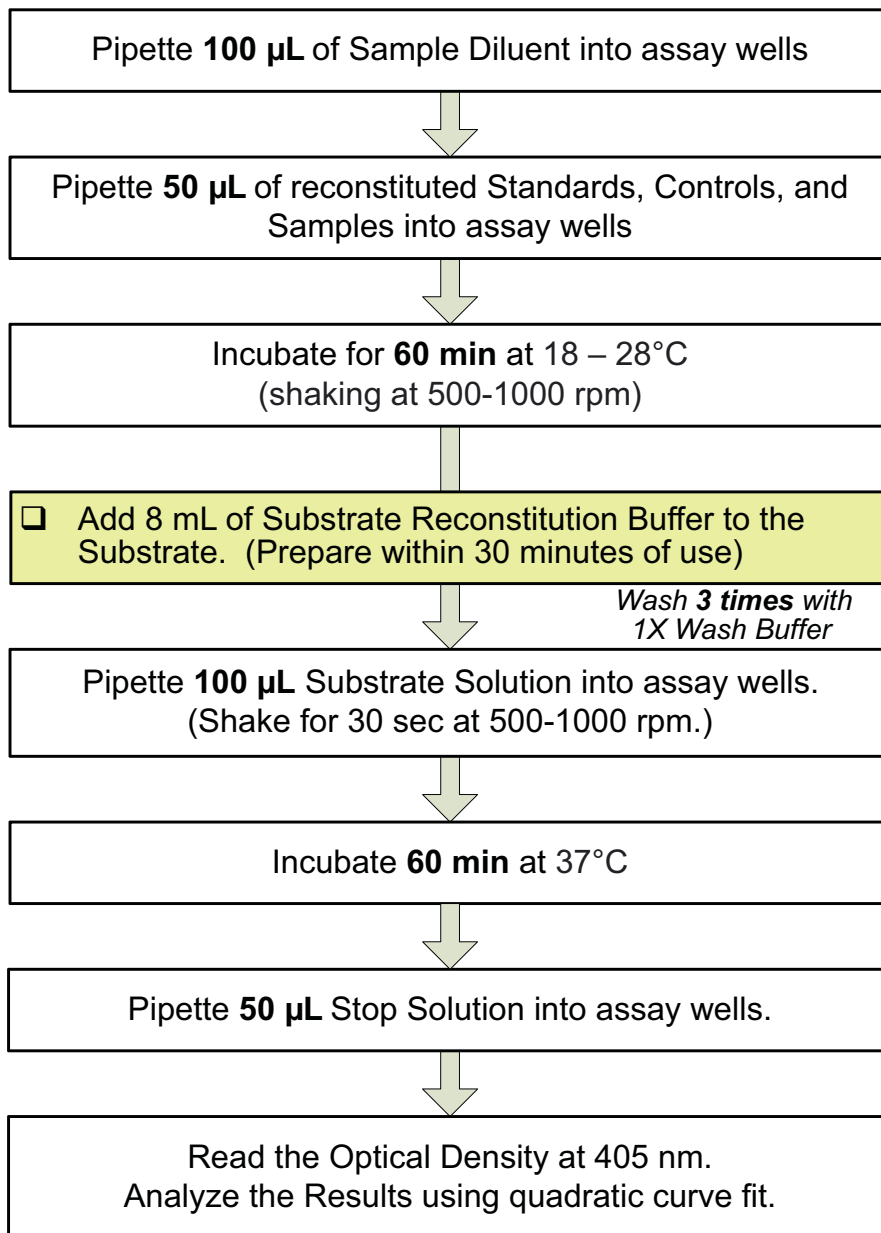
# MicroVue™ TRAP5b Enzyme Immunoassay Summary

## Standards and Controls Preparation

- Reconstitute Standards with 300  $\mu$ L of D.I. water. (Prepare Standards within 2 hours)
- Reconstitute Controls with 300  $\mu$ L of D.I. water. (Prepare Controls within 2 hours)
- Dilute 10X Wash Buffer 1:10 with D.I. water.

**NOTE: Mix Standards gently with pipette; do not vortex.**

## Assay Procedure



## INTENDED USE

The MicroVue TRAP5b Assay is an immunocapture enzyme assay for determination of tartrate-resistant acid phosphatase isoform 5b (TRAcP 5b). TRAP5b is secreted in serum by bone resorbing osteoclasts and is an indicator of osteoclast activity in vivo. Levels of TRAP5b activity may be a useful indicator of osteoclast activity and hence bone resorption in primary osteoporosis and other diseases.<sup>(5-10)</sup>

### Features

- The total assay time is two hours.
- The kit measures only active TRAP5b enzyme activity.
- Samples do not require pre-dilution.

### SUMMARY AND EXPLANATION

TRAP5b (serum band 5 tartrate-resistant acid phosphatase, TRAcP 5b; EC 3.1.3.2) is a 35-37 kDa glycoprotein. TRAP5b is typically expressed in proportion to osteoclast activity and is secreted into the circulation. Research indicates that serum TRAP5b is a potentially useful serological marker for bone resorption.<sup>9</sup>

The MicroVue TRAP5b Assay Kit detects the enzyme activity of TRAP5b based on an immuno-captured enzyme assay system.<sup>9</sup>

Elevated serum TRAP5b levels are thought to be associated with active bone remodelling. Increased serum levels are observed during normal bone growth among healthy children. Elevated serum TRAP5b levels have also been detected in certain disease states and conditions characterized by increased bone resorption.<sup>5, 14</sup> Examples are: Paget's disease of bone, hemodialysis, primary hyperparathyroidism, metastatic malignancies involving bone resorption, multiple myeloma, and bilaterally ovariectomized women. Post-menopausal women on estrogen replacement therapy typically have lower levels in serum than untreated postmenopausal women; therefore, specific determination of TRAP5b activity may be a potential means for the measurement and monitoring of changes in bone metabolism in response to therapy.

### PRINCIPLE OF THE PROCEDURE

The MicroVue TRAP5b Assay is a 2-step, direct capture, 96-well EIA. Serum or plasma samples and reconstituted Standards and Controls are added to coated microwell plate wells along with Sample Diluent.<sup>1-3</sup>

Naturally occurring, inactive TRAP5b fragments in the serum may interfere with the detection of TRAP5b in physiological samples. The MicroVue TRAP5b Assay avoids the influence of the inactive fragments by using two different monoclonal antibodies. The assay employs two unique monoclonal antibodies, Trk49 and Trk62, generated with immunization of purified TRAP5b from human bone cells. The first antibody, Trk49, is highly specific to inactive TRAP5b fragments; the second antibody, Trk62, is highly specific for intact, active TRAP5b. Trk49 binds inactive TRAP5b fragments, thereby making Trk62 more available to bind active TRAP5b in the microwell. The resulting TRAP5b assay is one that is specific and has good precision and wide range of linearity.

After the immunoreaction incubation, the plate is washed to remove unbound material, and a prepared substrate, 2-chloro-4-nitrophenyl phosphate (CNPP, pH 6.4), is added to the wells. Since the TRAP5b analyte is itself an enzyme, a labeled secondary antibody-enzyme conjugate is not required. At the end of this incubation, the reaction is stopped with the addition of a 0.2N NaOH solution and read via microplate reader at 405 nm. The TRAP5b activity is then calculated off a quadratic curve. The amount of color developed is proportional to the concentration of TRAP5b in the samples.

## REAGENTS AND MATERIALS PROVIDED

### 40 Assays for TRAP5b conducted in duplicate (96 wells)

MicroVue TRAP5b Assay kit contains the following:

<b>A</b>	<b>TRAP5b Standards:</b>	<b>Items 0711631-71</b>	<b>2 x 0.3 mL each</b>
<b>B</b>	(lyophilized) Human TRAP5b. The exact concentration is stated on each		
<b>C</b>	vial.		
<b>D</b>			
<b>E</b>			
<b>L</b>	<b>Controls</b>	<b>Items 0711681-91</b>	<b>2 x 0.3 mL each</b>
<b>H</b>	(lyophilized) Human TRAP5b. The concentration range is stated on the kit Certificate of Analysis (C of A).		
<b>1</b>	<b>Microwell Plate</b>	<b>Item 0711611</b>	<b>12 each</b>
	12 x 8 wells coated with murine monoclonal anti-TRAP5b antibodies		
<b>2</b>	<b>Stop Solution</b>	<b>Item 07116C1</b>	<b>8 mL</b>
	0.2N sodium hydroxide (NaOH)		
<b>3</b>	<b>10X Wash Buffer</b>	<b>Item 07116D1</b>	<b>40 mL</b>
	TBS/Tween. Contains 0.5% Tween® 20 and 0.02% ProClin® 300		
<b>4</b>	<b>Sample Diluent</b>	<b>Item 0711621</b>	<b>15 mL</b>
	Tris buffer. Contains 0.02% ProClin 300		
<b>5</b>	<b>Substrate Reconstitution Buffer</b>	<b>Item 07116B1</b>	<b>20 mL</b>
	MES buffer. Contains 0.02% ProClin 300		
<b>6</b>	<b>Substrate</b>	<b>Item 07116A1</b>	<b>2 x 8 mL</b>
	Substrate dissolving solution, 2-chloro-4-nitrophenyl-phosphate powder (CNPP)		
	<b>Plate Tape Cover</b>	<b>Item 0047</b>	<b>3 each</b>
	Tween® 20 is a registered trademark of ICI Americas Inc.		
	ProClin® is a registered trademark of Rohm and Haas Company.		

### MATERIALS REQUIRED BUT NOT PROVIDED

- Adjustable micropipettes for dispensing 50, 100, 300 µL, both single and multi-channel
- Microplate shaker capable of constant shaking at 500 – 1000 rpm for 60 minutes
- Incubator at 37°C
- Labware suitable for liquid measurement of 10-300 mL
- Deionized or distilled water
- Microplate reader capable of reading at 405 nm
- Computer with CD ROM Drive
- Software package facilitating data generation, quadratic curve fit, and data analysis
- Suitable device for washing the microplate
- Graduated pipette or equivalent for dispensing 8 mL
- Absorbent material for blotting the in-process microplate after washing

## WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This kit contains components of human origin. These materials were found to be non-reactive for HIV, HCV, and HBsAg. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
3. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
4. Dispose of containers and unused contents in accordance with National and Local regulatory requirements.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated.
7. Do not use Coated Strips if pouch is punctured.
8. Test each sample in duplicate.
9. Wear gloves and eye protection when handling contents of this kit. Use good laboratory practices to reduce exposure.
10. 0.2N NaOH acts as an irritant and can cause irritation to exposed areas. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
11. Avoid contact with the irritant Substrate Solution, which contains CNPP. In case of accidental contact, immediately wash skin thoroughly with soap and water.
12. ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Seek medical attention if symptoms are experienced.
13. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
14. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
15. Perform this assay with any validated washing method. Do not wash wells with a multi-channel pipette
16. Generate a standard curve with each assay.
17. Standard concentrations are assigned for each lot. Read label on each Standard vial or Certificate of Analysis carefully for specific concentrations.

## REAGENT PREPARATION

**All reagents should be equilibrated to 18-28°C prior to use.**  
Prepare assay reagents as follows:

### Sample Diluent

Sample Diluent is provided ready to use.

### Standards

Add 300 µL of deionized (distilled) water to the vial containing lyophilized Standard and dissolve for at least 5 minutes. Mix thoroughly. The reconstituted Standards should be used within 2 hours if stored at 18-28°C or within 24 hours if stored at 4°C.

## Controls

Add 300 µL of deionized (distilled) water to the vials containing lyophilized Controls, and dissolve for at least 5 minutes. Mix thoroughly. The reconstituted Controls should be used within 2 hours if stored at 18-28°C or within 24 hours if stored at 4°C.

## 10X Wash Buffer

Dilute 40 mL of 10X Wash Buffer with 360 mL deionized (distilled) water. The working Wash Buffer is stable for 1 month at 18-28°C.

## Substrate Solution

Prepare Working Substrate Solution by adding 8 mL of Substrate Reconstitution Buffer. Prepare within 30 minutes of use.

## Stop Solution

Stop Solution is provided ready to use.

## STORAGE

Store the kit at 2-8°C. Store unused reagents at 2-8°C. Under these conditions, assay components are stable until the expiry date printed on the kit label.

## SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (Heparin) can be used as samples in the MicroVue TRAP5b Assay. Collect serum using standard venipuncture technique, avoiding hemolysis. Allow the blood to clot, and separate the serum by centrifugation.

Samples can be stored up to 8 hours at room temperature, up to 2 days at 2-8°C, one month at -20°C, and at -80°C for long-term storage. Do not subject samples to more than 3 freeze/thaw cycles.

## ASSAY PROCEDURE

**Read entire product insert before beginning the assay.**

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

### Sample/Enzyme Incubation

1. Allow pouch of Coated Strips to equilibrate to 18-28°C before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch. Ensure that the pouch containing any unused strips is completely resealed and contains desiccant.
2. Pipette 100 µL of Sample Diluent into microplate wells.
3. Pipette 50 µL of each reconstituted Standard, Control and sample into appropriate microplate wells.
4. Seal the microwell plate with supplied plate tape cover and incubate for 60 minutes at 18-28°C on a microplate shaker set at 500 – 1000 rpm.
5. After incubation, wash the microplate wells three times with a minimum of 300 µL of Wash Buffer per well. After washing, tap the wells gently on a paper towel to expel any remaining liquid.

### Substrate Incubation

6. Pipette 100 µL of Working Substrate Solution into each well.
7. Seal the microplate and mix on a microplate shaker for 30 seconds at 500 – 1000 rpm. After shaking, incubate for 60 minutes in a 37°C incubator.

### Stop/Read

- Pipette 50  $\mu$ L of Stop Solution into each well to stop the reaction.
- Read and record the absorbance of each well at 405 nm.
- Use a quadratic curve fit for the standard curve. Calculate the values of Controls and specimens from the standard curve.

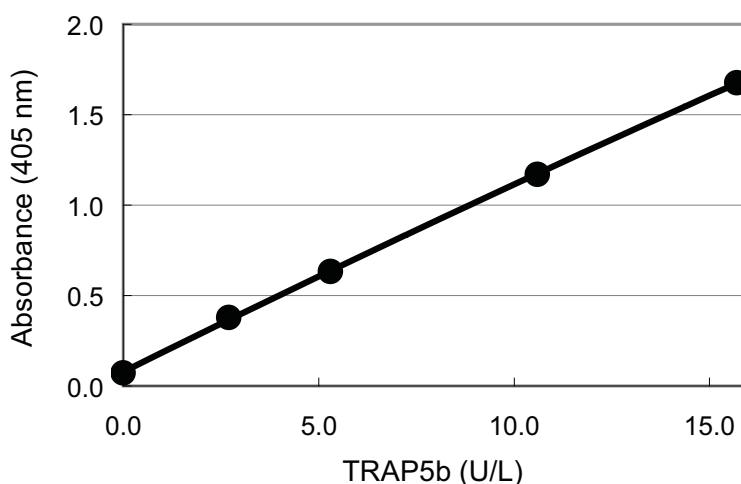
### QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values provided are intended as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be tested again.

### INTERPRETATION OF RESULTS

#### Representative Standard Curve



### OBSERVED VALUES

Observed serum values for TRAP5b activity in healthy men and women are reported as follows:

Gender	Age (years)	n	Mean (U/L)
Men	$\geq 20$	91	$4.0 \pm 1.4$
Women (Premenopausal)	30 - 44	31	$2.9 \pm 1.4$
Women (Postmenopausal)	$\geq 50$	36	$4.3 \pm 1.5$

Observed TRAP5b values (U/L) in 64 healthy adults (see gender and age information below) using both serum and plasma (Heparin) collection methods. Plasma samples were run for comparison to serum results.

- 28 Men, ages 25-54 (mean: 35.4)
- 36 Women, ages 21-59 (mean: 41.9)

Sample Type	Mean (U/L)	Min	Max	Correlation (r)
Serum	3.5 ± 1.4	1.2	6.7	–
Heparin Plasma	3.6 ± 1.4	1.2	7.3	0.989

## PERFORMANCE OF THE TEST

Typical analytical data of MicroVue TRAP5b Assay are presented in this section. For kit lot-specific standard curve and controls values see the Certificate of Analysis.

### Sensitivity

The minimum detection limit of the MicroVue TRAP5b assay is 0.2 U/L, determined by the upper 3 SD limit in a zero standard precision study.

### Precision

- Intra assay (Within Run) (n = 16)

Sample	Mean (U/L)	Standard Deviation (U/L)	%CV
1	3.4	0.07	2.2
2	7.4	0.14	1.9

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

Sample	Mean (U/L)	Standard Deviation (U/L)	%CV
1	3.8	0.11	3.0
2	7.4	0.15	2.0

### Spike Recovery

Spike recovery of 92-103% was determined by adding a known quantity of purified TRAP5b to serum samples with different levels of endogenous TRAP5b.

## Linearity

Linearity was performed by serially diluting serums with sample diluent and comparing observed values with expected values.

Sample	Dilution Factor	Observed (U/L)	Expected (U/L)	Recovery (%)
1	neat	3.7	-	-
	1:2	1.8	1.8	95.9
	1:4	0.9	0.9	95.1
	1:8	0.5	0.5	101.2
2	neat	7.7	-	-
	1:2	3.8	3.8	99.8
	1:4	1.9	1.9	97.5
	1:8	0.9	1.0	97.4
3	neat	12.0	-	-
	1:2	5.8	6.0	96.2
	1:4	3.0	3.0	100.8
	1:8	1.4	1.5	95.9

## Interfering Substances

The following substances were tested at the specified concentrations and were found not to interfere with the assay:

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin F	20 mg/dL
Bilirubin C	20 mg/dL
Lipids (Intralipid®)	2500 Turbidity
RF (Rheumatoid Factor)	500 U/mL

Intralipid® is a registered trademark of Fresenius Kabi AB.

## CUSTOMER ASSISTANCE

To place an order or for technical assistance, please contact your local distributor.

Additional information about Quidel and Quidel's products and distributors can be found on our website at [www.quidel.com](http://www.quidel.com).

## REFERENCES

1. Igarashi Y, Mochizuki Y, Miura T, Ohashi T, Sasagawa K, Katayama K, Inaba N, Matsuzaki S. Evaluation of a novel immunoassay for serum tartrate-resistant acid phosphatase type 5b activity in hormone replacement therapy. *Bone* 2003; 32(5): S179.
2. Minkin C. Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. *Calcif Tissue Int.* 1982, 34, 285-290.
3. 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.
4. 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.
5. 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.
6. Halleen JM, Alatalo SL, Janckila AJ, Woitge HW, Seibel MJ, Väänänen HK 2001 Serum Tartrate-resistant acid phosphatase is a specific and sensitive marker of bone resorption. *Clin Chem.* 47:597-600.
7. Halleen JM 2003 Tartrate-resistant acid phosphatase 5B is a specific and sensitive marker of bone resorption (Review). *Anticancer Res.* 23(2A):1027-1029.
8. Janckila AJ, Takahashi K, Sun SZ, Yam LT 2001 Tartrate-resistant acid phosphatase isoform 5b as serum marker for osteoclastic activity. *Clin Chem.* 47:74-80.
9. Lamp EC, Drexler HG. Biology of tartrate-resistant acid phosphatase. *Leuk Lymphoma.* 2000, 39, 477-484.
10. Leeming, et al 2006 The relative use of eight collagenous and noncollagenous markers for diagnosis of skeletal metastases in breast, prostate or lung cancer patients, *Cancer epidemiology Biomarkers.* 15(1).
11. 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.
12. 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.
13. Igarashi Y, Lee M, Matsuzaki S. Acid phosphatases as markers of bone metabolism. *J Chromatogr B.* 2002, 781, 345-358.
14. 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 marker for monitoring bone disease in multiple myeloma. *Int J Cancer.* 2003, 106, 455-457.



Catalog Number



Manufacturer



Contains sufficient for  
<n> tests



Consult Instructions for Use



Authorized Representative  
in the European Community



Temperature Limitation



*In Vitro* Diagnostic  
Medical Device



Contents / contains



Intended Use



Instructions for use on CDROM



Biological risks



**REF** 8036 – MicroVue™ TRAP5b Enzyme Immunoassay Kit



MDSS GmbH  
Schiffgraben 41  
30175 Hannover, Germany



Nitto Boseki Co., Ltd.  
Fukushima, Japan

for:

**QUIDEL**<sup>®</sup>  
CORPORATION  
SPECIALTY PRODUCTS  
RESEARCH TO RAPIDS<sup>®</sup>

Quidel Corporation | 10165 McKellar Court  
San Diego, CA 92121 USA | [www.quidel.com](http://www.quidel.com)

0961B (2009/07)