



MicroVue™ Bone

TRAP5b EIA

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

For *In Vitro* Diagnostic Use. For export only. Not for sale or use in the United States or Canada.

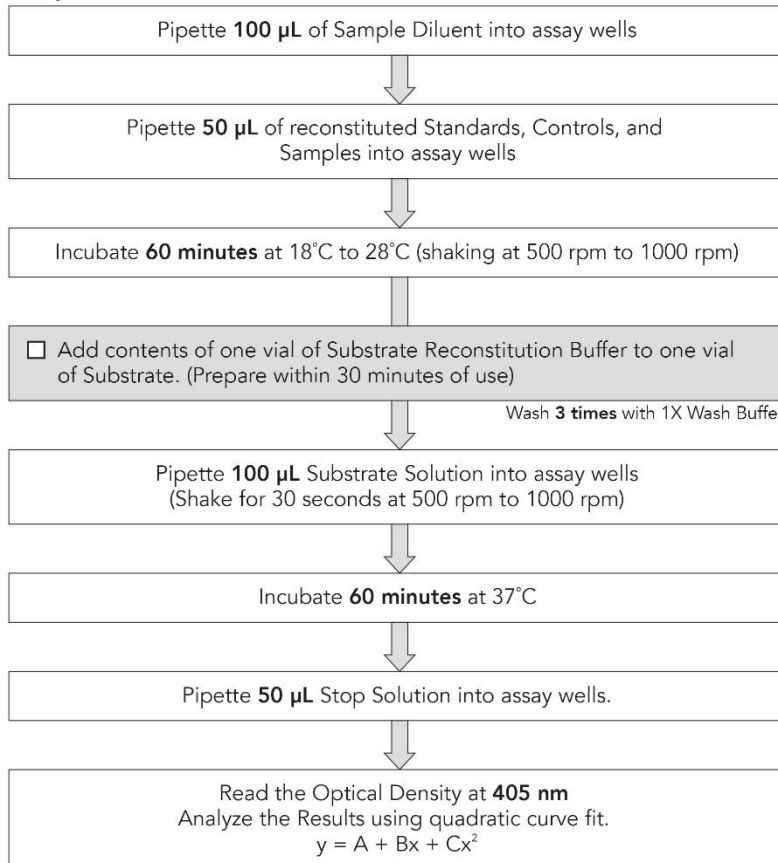
SUMMARY

Standards and Controls Preparation

- Reconstitute Standards with 400 µL of DI water. (Prepare Standards within 2 hours)
- Reconstitute Controls with 400 µL of DI water. (Prepare Controls within 2 hours)
- Dilute 10X Wash Buffer 1:10 with DI 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) in human serum or plasma. 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.¹⁻⁶

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.⁵

The MicroVue TRAP5b Assay Kit detects the enzyme activity of TRAP5b based on an immuno-captured enzyme assay system.⁵

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.^{1, 14} 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.⁷⁻⁹

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:

A	TRAP5b Standards: (lyophilized) recombinant protein composed of Human TRAP5b. The exact concentration is stated on E each vial	Parts 0711631-71	0.4 mL, 2 each
L	Low/High Controls H (lyophilized) recombinant protein composed of Human TRAP5b. The concentration range is stated on the kit Certificate of Analysis (C of A)	Parts 0711681-91	0.4 mL, 2 each
1	Microwell Plate 12 x 8 wells coated with murine monoclonal anti-TRAP5b antibodies	Part 0711611	12 each
2	Stop Solution 0.2N sodium hydroxide (NaOH)	Part 07116C1	12 mL
3	10X Wash Buffer TBS/Tween. Contains 0.5% Tween® 20 and 0.02% ProClin® 300	Part 07116D1	100 mL
4	Sample Diluent Tris buffer. Contains 0.02% ProClin 300	Part 0711621	20 mL
5	Substrate Reconstitution Buffer MES buffer. Contains 0.02% ProClin 300	Part 07116B1	12 mL, 2 each
6	Substrate Substrate dissolving solution, 2-chloro-4-nitrophenyl-phosphate powder (CNPP)	Part 07116A1	12 mL, 2 each
	Plate Tape Cover	Part 0047	3 each

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
- Software package facilitating data generation, quadratic curve fit, and data analysis
- Suitable device for washing the microplate
- Graduated pipette or equivalent for dispensing 12 mL
- Absorbent material for blotting the in-process microplate after washing

WARNINGS AND PRECAUTION

- For *in vitro* diagnostic use
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.

- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- Test each sample in duplicate.
- Wear gloves and eye protection when handling contents of this kit. Use good laboratory practices to reduce exposure.
- 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.
- Avoid contact with the irritant Substrate Solution, which contains CNPP. In case of accidental contact, immediately wash skin thoroughly with soap and water.
- 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.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
- For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
- Perform this assay with any validated washing method. Do not wash wells with a multi-channel pipette
- Generate a standard curve with each assay.
- Standard concentrations are assigned for each lot. Read label on each Standard vial or Certificate of Analysis carefully for specific concentrations.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

Store the kit at 2°C to 8°C. Store unused reagents at 2°C to 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°C to 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.

REAGENT PREPARATION

All reagents should be equilibrated to 18°C to 28°C prior to use.

Prepare assay reagents as follows:

Sample Diluent

Sample Diluent is provided ready to use.

Standards

Add 400 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°C to 28°C or within 24 hours if stored at 4°C.

Controls

Add 400 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°C to 28°C or within 24 hours if stored at 4°C.

10X Wash Buffer

Dilute 100 mL of 10X Wash Buffer with 900 mL deionized (distilled) water. The working Wash Buffer is stable for 1 month at 18°C to 28°C.

Substrate Solution

Prepare Working Substrate Solution by adding the contents of one vial of Substrate Reconstitution Buffer to the contents of 1 vial of Substrate. Prepare within 30 minutes of use.

Stop Solution

Stop Solution is provided ready to use.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

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

Determine amount of each reagent required for the number of strips to be used.

# of Strips	4	6	8	12
# of Samples (tested in duplicate)	8	16	24	40
Substrate (vial)	1	1	1	1
1X Wash Buffer (mL)	100	150	200	300

Sample/Enzyme Incubation

1. Allow pouch of Coated Strips to equilibrate to 18°C to 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°C to 28°C on a microplate shaker set at 500 rpm to 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 μ 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.

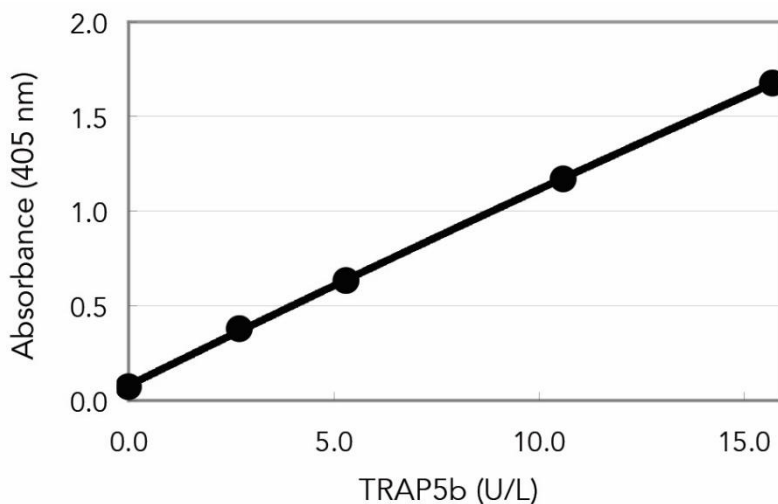
QUALITY CONTROL

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.

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	≥ 20	91	4.0 ± 1.4
Women (Premenopausal)	30 to 44	31	2.9 ± 1.4
Women (Postmenopausal)	≥ 50	36	4.3 ± 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 to 54 (mean: 35.4)
- 36 Women, ages 21 to 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% to 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.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF

8036 – MicroVue TRAP5b EIA Kit

IVD



EC REP

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PI8036000EN00 (02/17)

GLOSSARY

REF

Catalogue number



CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code



Use by



Manufacturer



Temperature limitation



Intended use



Consult e-labeling
instructions for use



Biological risks

IVD

For *In Vitro* diagnostic use



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control
