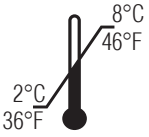
MicroVue™ Rat TRAP5b EIA Kit

Bone Health

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

For Research Use Only.
Not for Use in Diagnostic Procedures.



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REF 8042

Rat TRAP5b Enzyme Immunoassay Summary

Standards and Controls Preparation

- ❑ Reconstitute Standards with 500 µL of D.I. water. (Prepare Standards within 2 hours)
- ❑ Reconstitute Controls with 500 µL 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

Pipette **100 µL** of Sample Diluent into assay wells

↓

Pipette **20 µL** of reconstituted Standards, Controls, and Samples into assay wells

↓

Incubate for **60 min** at 20 – 25°C
(shaking at 500-1000 rpm)

↓

❑ Add contents of 1 vial of Substrate Reconstitution Buffer to the Substrate. *(Prepare within 30 minutes of use)*

↓

*Wash **3 times** with 1x Wash Buffer*

Pipette **100 µL** Substrate Solution into assay wells.
(Shake for 30 sec at 500-1000 rpm.)

↓

Incubate **90 min** at 37°C

↓

Pipette **50 µL** Stop Solution into assay wells.

↓

Read the Optical Density at 405 nm using a 490 nm reference filter. Analyze the Results using a quadratic curve fit. $y = (A-D)/(1+(x/C)^B)+D$

PURPOSE OF THE TEST

The MicroVue Rat TRAP5b Assay is an immunocapture enzyme assay for determination of tartrate-resistant acid phosphatase isoform 5b (TRAcP 5b). Rat TRAP5b is derived from osteoclasts in rat serum. This kit is intended for research use only.

Features

- The total assay time is 2.5 hours.
- The kit measures only active Rat TRAP5b enzyme activity.
- Samples do not require pre-dilution.

SUMMARY AND EXPLANATION

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

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

PRINCIPLE OF THE PROCEDURE

The MicroVue Rat TRAP5b Assay is a 2-step, direct capture, 96-well EIA. Serum samples and reconstituted Standards and Controls are added to coated microwell plate wells along with Sample Diluent.

The capture antibody, Trk49R, is highly specific for intact, active Rat TRAP5b.

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 Rat 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 405/490 nm. The Rat TRAP5b activity is then calculated off a quadratic curve. The amount of color developed is proportional to the concentration of Rat TRAP5b in the samples.

REAGENTS AND MATERIALS PROVIDED

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

MicroVue Rat TRAP5b Assay kit contains the following:

A **Rat TRAP5b Standards: Items 0711632-72 2 x 0.5 mL each**
B (lyophilized) Rat TRAP5b. The exact concentration is stated on each vial
C
D
E

L **Controls Items 0711682-92 2 x 0.5 mL each**
H (lyophilized) Rat TRAP5b. The concentration range is stated on the kit Certificate of Analysis (C of A)

❶ **Microwell Plate Item 0711612 12 each**
12 x 8 wells coated with murine monoclonal anti-Rat TRAP5b antibodies

❷ **Stop Solution Item 07116C2 12 mL**
0.2N sodium hydroxide (NaOH)

❸ **10X Wash Buffer** **Item 07117D2** **50 mL**
TBS/Tween. Contains 0.5% Tween® 20 and 0.02% ProClin® 300

❹ **Sample Diluent** **Item 0711722** **15 mL**
Tris buffer. Contains 0.02% ProClin 300

❺ **Substrate Reconstitution Buffer** **Item 07117B2** **2 x 8 mL**
MES buffer. Contains 0.02% ProClin 300

❻ **Substrate** **Item 07117A2** **2 x 8 mL**
Substrate dissolving solution, 2-chloro-4-nitrophenyl-phosphate powder (CNPP)

Plate Tape Cover **Item 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 20, 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 with a 490 nm reference filter
- Computer with Software package for 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. Research Use Only. Not for Use in Diagnostic Procedures.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any biological samples.
3. Dispose of containers and unused contents in accordance with National and Local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use Coated Strips if pouch is punctured.
7 For best results, test each sample in duplicate.
8. Wear gloves and eye protection when handling contents of this kit. Use good laboratory practices to reduce exposure.
9. 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.
10. Avoid contact with the irritant Substrate Solution, which contains CNPP. In case of accidental contact, immediately wash skin thoroughly with soap and water.

11. 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.
12. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
13. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
14. Perform this assay with any validated washing method. Do not wash wells with a multi-channel pipette
15. Generate a quadratic standard curve with each assay.
16. Standard concentrations are assigned for each lot. Read the 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 500 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 20-25°C or within 24 hours if stored at 4°C.

Controls

Add 500 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 20-25°C or within 24 hours if stored at 4°C.

10X Wash Buffer

Dilute 50 mL of 10X Wash Buffer with 450 mL deionized (distilled) water. The working Wash Buffer is stable for 1 month at 20-25°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 just prior to 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

Rat serum is the only sample type to be used in the MicroVue Rat 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 to -30°C, and at -80°C for 24 months. 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*.

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	2*	2*
1X Wash Buffer (mL)	100	150	200	300

* When more than one bottle or vial is to be used, combine the contents and mix prior to use.

Sample/Enzyme Incubation

1. Allow pouch of Coated Strips to equilibrate to 20-25°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 20 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 20-25°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 90 minutes in a 37°C incubator.

Stop/Read

8. Pipette 50 µL of Stop Solution into each well to stop the reaction.
9. Read and record the absorbance of each well at 405 nm using a 490 nm reference filter.
10. Use a quadratic curve fit for the standard curve. Calculate the values of Controls and specimens from the standard curve.

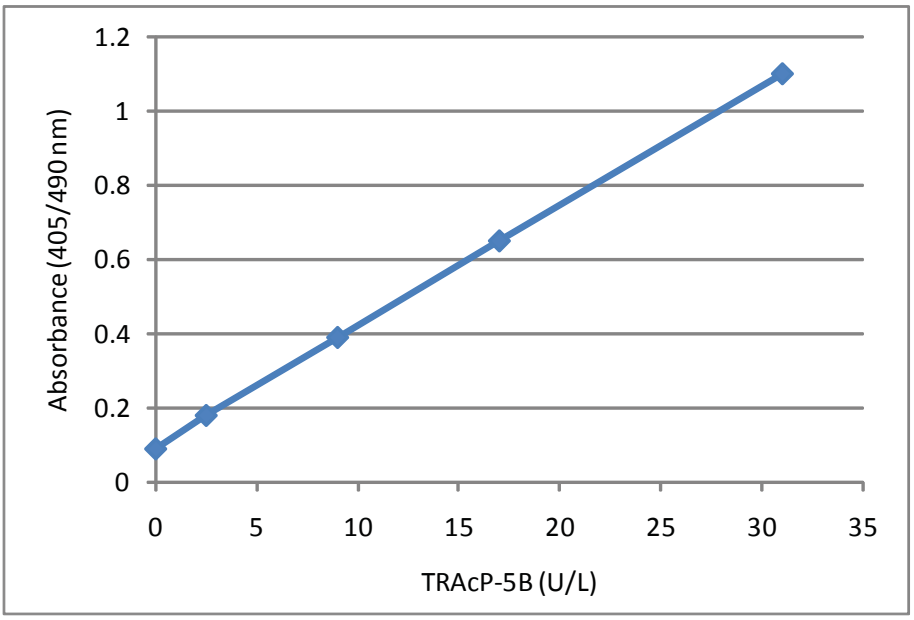
QUALITY CONTROL

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 Rat TRAP5b activity in healthy female subjects are reported as follows:

Group	Age (weeks)	n	Mean (U/L)
Wister, female	8	10	19.47
	12	10	15.40

PERFORMANCE OF THE TEST

Sensitivity

The minimum detection limit of the MicroVue Rat TRAP5b assay is 0.4 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	10.80	0.25	2.2
2	18.77	0.55	2.8

- Inter assay (Run to Run) (n = 8 reps over 7 days)

Sample	Mean (U/L)	Standard Deviation (U/L)	%CV
1	10.92	0.15	1.4
2	18.69	0.51	2.7

CUSTOMER ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

Additional information about Quidel and Quidel's products and distributors can be found on our website at www.quidel.com.

REFERENCES

1. Altalo SL, Halleen JM, Hentunen TA, Monkkonen J, Vaananen HK. Rapid screening method for osteoclast differentiation in vitro that measures tartrate resistant acid phosphatase 5b activity secreted into the culture medium. *Clin Chem*. 2000, 46, 1751-1754.
2. Altalo SL, Peng Z, Janckila AJ, Kaija H, Vihko P, Vaananen HK, Halleen JM. A novel immunoassay for the determination of tartrate-resistant acid phosphatase 5b from rat serum. *J Bone Miner Res*. 2003, 18, 134-139.
3. Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M, Kostenuik PJ, Giannobile WV. RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodonitis. *J Periodontol.* 2007, 78, 1300-1308.
4. Kikuchi W, Miura T, Ito K, Sasagawa K, Namatame Y, Igarashi Y, Oda K, Kojima R. Development of a new assay kit for tartrate-resistant acid phosphatase type 5b in rat serum. Abstract of poster presentation at *ASBMR 31th Annual Meeting 2009* (Denver). 2009.
5. Rani S, Barbe MF, Barr AE, Litvin J. Periostin-like-factor and periostin in an animal model of work-related musculoskeletal disorder. *Bone.* 2009, 44, 502-512.
6. Sample SJ, Collins RJ, Wilson AP, Racette MA, Behan M, Markel MD, Kalscheur VL, Hao Z, Muir P. Systemic effects of ulna loading in maile rats during functional adaptaion. *J Bone Mine Res*. 2010, 25, 2016-2028.
7. Chow DH-K Leung K-S, Qin L, Leung AH-C, Cheung W-H. Low-magnitude high-frequency vibration (LMHFV) enhances bone remodeling in osteoporotic rat femoral fracture healing. *J Orthop Res.* 2011, 29, 746-752.

Consult Instructions for Use

Manufacturer

Catalog Number

Contents / contains

Contains sufficient for <n> tests

Temperature Limitation

Instructions for use on CDROM

Biological risks

REF **8042 – MicroVue™ Rat TRAP5b Enzyme Immunoassay Kit**

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