BoneTRAP® Assay

SB-TR201A

English/Français/Deutsch/Español/Italiano

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研究用

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BoneTRAP® Assay

Test for the quantitative determination of the active isoform 5b of the tartrate-resistant acid phosphatase (TRACP)

Cat. no.: SB-TR201A

For in vitro diagnostic use only

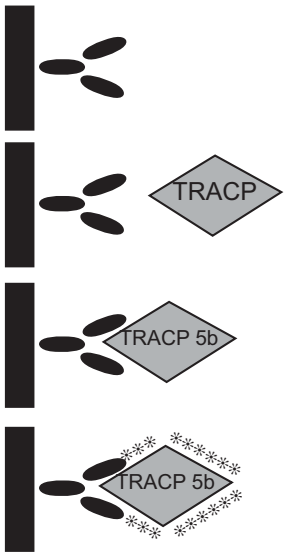
Introduction

High amount of tartrate-resistant acid phosphatase (TRACP) is expressed by bone-resorbing osteoclasts and activated macrophages (1). Two forms of TRACP circulate in human blood, known as TRACP 5a and TRACP 5b (2). TRACP 5b is derived from osteoclasts and TRACP 5a from macrophages (3).

Osteoclasts secrete TRACP 5b into the blood circulation as an active enzyme that is inactivated and degraded to fragments before it is removed from the circulation. Thus, TRACP 5b activity does not accumulate into the circulation in renal or hepatic failure (4,5). All serum TRACP 5b activity is derived from osteoclasts. Diurnal variability of serum TRACP 5b activity is low and the levels are not affected by feeding, allowing sample collection at any time of day (5).

The BoneTRAP® assay is a specific method to detect TRACP 5b activity freshly liberated from osteoclasts. It is intended for use as an indicator of bone resorption and can be used as an aid in monitoring bone resorption changes in post-menopausal women and individuals diagnosed with osteoporosis undergoing anti-resorptive therapies (HRT and bisphosphonates) (4, 6-18). *In vitro*, TRACP 5b activity reflects the number of osteoclasts (15,19), and therefore the BoneTRAP® Assay can be conveniently used to determine osteoclast number in human osteoclast cultures.

Test principle



The plate is coated with anti-TRACP antibodies (monoclonal).

Calibrators, Control and patient samples are added.
Releasing reagent is added.
Dissociation of active TRACP 5b from the binding proteins.

TRACP 5b is bound by the anti-TRACP antibodies.

Incubation with pNPP substrate (*).

The reaction is stopped by adding sodium hydroxide. The absorption is read photometrically.

Advantages of the test

- Measures TRACP 5b activity that is released specifically from osteoclasts.
- No interference with TRACP 5a or other phosphatases.
- Hemolysis has no effect on results.
- No diurnal variation.
- Not affected by functional disorders of kidney and liver.
- No dietary influences.

KIT CONTENTS

Cat. no.: SB-TR201A

1. **MICROPLAT** Microplate: 12 x 8 wells (with frame and desiccant in aluminium bag), F-form, coated with monoclonal anti-TRACP antibody (mouse) and BSA, ready to use.

2. **CTRL** Controls: 2 x 2 vials with 0.5 ml each, human recombinant TRACP, lyophilized, Xn, Harmful, R 22-52/53, S 36-60, contains < 1 % sodium azide and BSA.

3. **CAL** Calibrators: 2 x 6 vials with 0.5 ml each, human recombinant TRACP, lyophilized, Xn, Harmful, R 22-52/53, S 36-60, contains < 1 % sodium azide and BSA. The exact value of each calibrator is printed on the bottle label.

4. WASHBUF 25X Wash Buffer: 1 bottle with 40 ml TBS/Tween (25x conc.), pH 7.65 – 7.85, contains < 1 % Germall®II.

5. SAMPDIL Sample Diluent: 1 vial with 15 ml, sodium chloride solution, ready to use, contains < 1 % Germall®II.

6. RELEASREAG Releasing Reagent: 1 vial with 8 ml, pH 6.9 – 7.1, ready to use, contains < 1 % Germall®II.

7. SUBSBUF Substrate Buffer: 2 vials with 10 ml each, sodium acetate buffer, pH 5.95 – 6.05, ready to use, contains < 1 % Germall®II.

8. SUBS pNPP Substrate Tablets: 4 tablets, contain p-nitrophenyl phosphate (pNPP).

9. NaOH Stop Solution: 1 vial with 6 ml, 0.32 M sodium hydroxide, ready to use, Xi, Irritant, R 36/38, S 26-37-60.

1. STORAGE AND STABILITY

MATERIAL/REAGENT	STATE	STORAGE	STABILITY
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Control	reconstituted	-18 °C or below	6 weeks
Calibrators	reconstituted	-18 °C or below	6 weeks
Substrate Buffer	opened	2...8 °C	6 weeks
Wash Buffer	diluted	2...8 °C	6 weeks
Sample Diluent	opened	2...8 °C	6 weeks
Releasing Reagent	opened	2...8 °C	6 weeks
Stop Solution	opened	2...8 °C	6 weeks

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of Wash Buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37 °C.

2.6. Microplate shaker, shaking frequency 850–950 rpm, amplitude 4 mm.

2.7. Microplate reader with filter for 405 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate
The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated in table 1.

3.2. Wash Buffer
Mix one volume of Wash Buffer (25x) with 24 volumes of water for injection (e.g. 10 ml Wash Buffer (25x) with 240 ml water). Seven ml of diluted wash buffer is needed for 8 wells.

3.3. Calibrators
Reconstitute the lyophilised calibrators each with 0.5 ml of water for injection. Reconstitution time 15 min.

3.4. Controls
Reconstitute the lyophilised controls with 0.5 ml of water for injection. Reconstitution time 15 min.

3.5. Substrate Solution
1 Substrate Tablet is dissolved in 5 ml Substrate Buffer.
The Substrate Solution must not be stored.

Do not mix reagents from different lots or manufacturers.

Valid and reproducible results are only obtained if the test procedure is precisely followed and test kit-specific reagents are used.

. SPECIMEN

4.1. The assay is suitable for serum and EDTA-plasma samples.
NB. The same specimen type must be used throughout a follow-up study.

4.2. Pretreatment of sera, e.g. inactivation, must not be performed. The specimen should not be contaminated with microorganisms.

4.3. Specimen can be stored up to 8 hours at room temperature and up to 3 days at 2-8 °C. Storage at –20 °C is possible for 2 months. For longterm storage a temperature of –80 °C is necessary.

4.4. Samples are used undiluted. Specimen above the measuring range can be diluted up to 1:5.

5.A. TEST PROCEDURE

5.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be pre-washed.
NB! Microplate 12x8 wells.

5.2. Add 100 µl each of Calibrators, Control and samples to the wells of the plate in duplicate.

5.3. Add 50 µl Releasing Reagent to each well.

5.4. Seal the microplate with incubation cover foil and incubate for 60 min (± 5 min) at room temperature with constant shaking at 850–950 rpm.

5.5. After incubation wash the microplate wells four times with 300 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.6. Add 100 µl Substrate Solution to each well.

5.7. Seal the microplate with incubation cover foil and incubate for 60 min (± 5 min) at 37 °C (± 1 °C).

5.8. Stop the reaction by adding 25 µl of Stop Solution to each well.

Ensure for a good mixing by shaking gently.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the Stop Solution!

5.B. TABLE FOR THE TEST PROCEDURE

	Calibrators	Control	Sample
		-	
Calibrators	100 µl	-	-
Controls	-	100 µl	-
Sample	-	-	100 µl
Releasing Reagent	50 µl	50 µl	50 µl
Incubate for 60 min (± 5 min) at room temperature with constant shaking at 850–950 rpm, wash 4 x with 300 µl wash buffer.			
Substrate Solution	100 µl	100 µl	100 µl
Incubate for 60 min (± 5 min) at 37 °C (± 1 °C).			
Stop Solution	25 µl	25 µl	25 µl
Photometric reading at 405 nm			

6.A. CALCULATION OF RESULTS (VALIDITY)

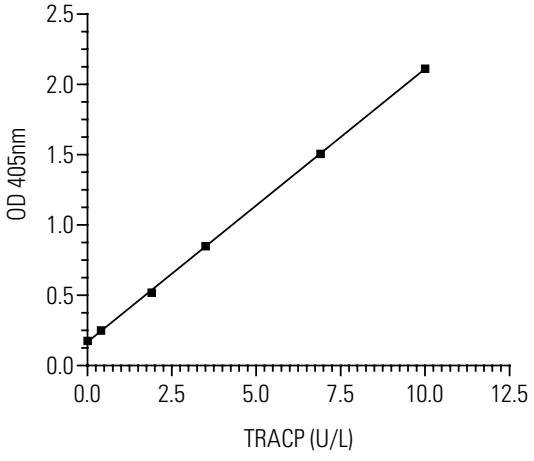
* Read OD values at 405 nm.

* The average OD values of the calibrators are plotted against the activity values. The calibration line is calculated by linear regression.

* The measuring range spans from 0.5 to 10 U/L. Samples below the measuring range have to be interpreted as < 0.5 U/L. Samples with activities above the measuring range have to be interpreted as > 10 U/L. These values must not be extrapolated but the samples should be retested diluted (up to 1:5).

* The TRACP 5b activities of the controls and the samples can be read from the calibration line. If diluted samples have been used the dilution factor has to be considered.

Example for calibration line:



* Lot-specific data
  Refer to Quality Control Report.

* Validity criteria
  - The average OD of the 0 U/L calibrator has to be < 0.400.
  - The activity of the control has to be within the nominal range. Refer to Quality Control Report for assigned range.
  - The correlation coefficient (r^2) of the calibration line has to be ≥ 0.99.

Repeat the run if the results do not meet the specification!

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Increased TRACP 5b activity (see 7.E.) indicates an increased bone resorption.
* Results within the reference range do not exclude disorders in bone metabolism completely. Therefore all results should always be interpreted in connection with clinical data and additional diagnostic parameters.
* High concentrations of hemoglobin do not influence the test results.
* High lipid concentrations may reduce the OD values and distort the TRACP activity.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the evaluation of the assay.

7.A. PRECISION

Sample	**Intra-assay variation** (n = 21)			**Sample**	**Inter-assay variation** (n = 11)		
	mean U/L	**SD**	**CV (%)**		**mean U/L**	**SD**	**CV (%)**
Control	3.0	0.18	6.0	**Control**	3.3	0.19	5.8
N° 1	2.6	0.25	9.6	**N° 1**	2.5	0.23	9.2
N° 2	3.1	0.43	13.9	**N° 2**	4.2	0.35	8.3
N° 3	7.1	0.47	6.6	**N° 3**	7.0	0.62	8.9
				N° 4	7.2	0.38	5.4
				N° 5	2.6	0.23	8.8
				N° 6	16.1	1.16	7.2

7.B. RECOVERY

By adding 3 defined TRACP 5b activities each to 3 different sera a mean recovery of 100.9 % (SD = 11.3 %) was determined.

7.C. DILUTION LINEARITY

A linearity was determined by using sera of different activity (n=5). Samples with high TRACP 5b activity can be diluted up to 1:5 with Sample Diluent.

7.D. LIMIT OF QUANTITATION

The limit of quantitation is < 0.5 U/L.

7.E. EXPECTED VALUES

Expected values of TRACP 5b were determined from the serum of 239 healthy blood donors as follows:

Group	n	Mean Age (range)	TRACP 5b (U/L) Mean ± SD
Healthy premenopausal women	144	39.5 (22 – 54)	2.59 ± 0.78
Healthy young men	32	36.0 (22 – 54)	3.06 ± 0.88
Healthy postmenopausal women	46	60.3 (41 – 81)	3.19 ± 0.85
Healthy old men	17	68.5 (55 – 79)	3.31 ± 0.72

Upper limits of normal were calculated as the mean + 2 SD of premenopausal women (for women) and young men (for men):

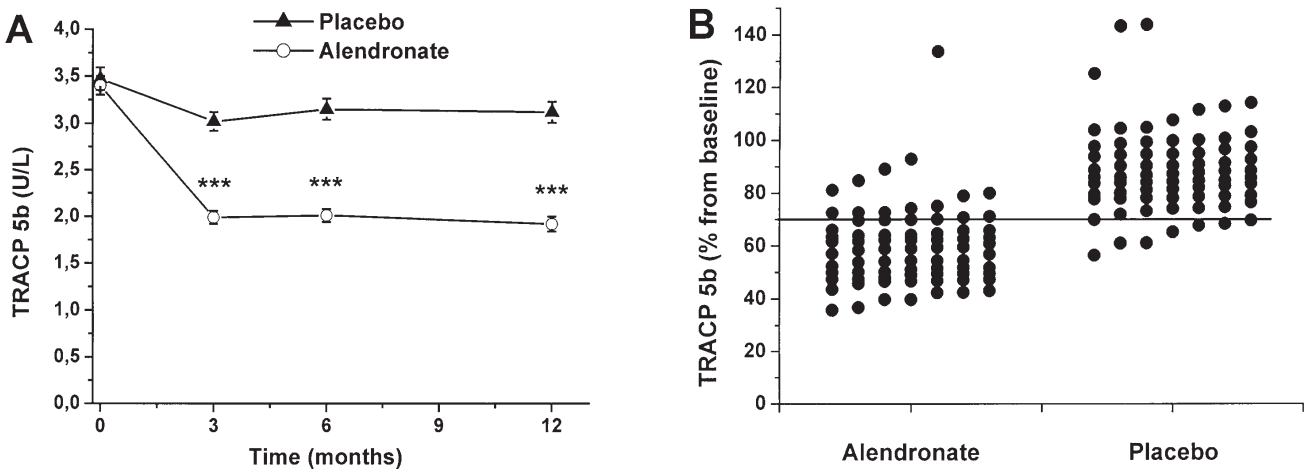
Group	Upper normal limit (mean + 2SD)
Women	4.15 U/L
Men	4.82 U/L

8. CLINICAL IMPORTANCE

TRACP 5B ACTIVITY IN POSTMENOPAUSAL WOMEN UNDER ALENDRONATE THERAPY

(see ref. 14)

TRACP 5b values were determined from the serum of postmenopausal women receiving 5 mg alendronate daily for 12 months in a placebo-controlled study. All subjects in the placebo (n = 73) and alendronate (n = 75) groups received a daily supplement of 630 mg calcium carbonate and 200 IU vitamin D.



A) Serum TRACP 5b activity (U/L) before the start of treatment (0) and at 3, 6 and 12 months; B) The change of serum TRACP 5b at 3 months. Each spot shows the value of one individual at 3 months compared with the value obtained for the same individual at baseline (before the start of treatment). The line in B) shows the Least Significant Change (LSC = 29.5%). A decrease of more than LSC was observed for 82.7% of the individuals in the alendronate group, and 11.0% of the individuals in the placebo-group.

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of animal origin (see kit contents) should be handled as potentially infectious and used with all necessary precautions.

* R 22: Harmful if swallowed.
  R 36/38: Irritating to eyes and skin.
  R 52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
  S 26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
  S 36: Wear suitable protective clothing.
  S 37: Wear suitable gloves.
  S 60: This material and its container must be disposed of as hazardous waste.

Wash Buffer, Sample Diluent, Releasing Reagent and Substrate Buffer contain Germall®II (diazolidinyl urea): May produce an allergic reaction.

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

BoneTRAP® Assay

Test pour la détermination quantitative de l'isoforme active 5b de la phosphatase acide tartrate résistante (TRACP)

Cat. no.: SB-TR201A
Usage diagnostique in vitro.

Introduction

La Phosphatase Acide Osseuse résistante au tartrate (TRACP) est exprimée par les ostéoclastes et par l'activation des macrophages (1). Deux formes de la TRACP circulent dans le sang humain : la TRACP 5a et la TRACP 5b (2). La TRACP 5b est dérivée des ostéoclastes, la TRACP 5a des macrophages (3).

Les ostéoclastes secrètent la TRACP 5b dans la circulation sanguine en forme d'une enzyme active qui est inactivée et dégradée en fragments avant d'être supprimée de la circulation. Par conséquent, l'activité de la TRACP 5b ne s'accumule pas dans la circulation en cas d'insuffisance rénale ou hépatique (4,5). Toute l'activité de la TRACP 5b dans le sérum est dérivée des ostéoclastes. La variabilité diurnale de l'activité de la TRACP 5b sérique est basse et les niveaux ne sont pas affectés par l'alimentation, ce qui permet de collecter les échantillons à toute heure de la journée (5).

L'essai BoneTRAP® est une méthode spécifique pour détecter l'activité de la TRACP 5b libérée des ostéoclastes. Le test peut ainsi déterminer un taux de résorption osseuse anormal chez les sujets atteints d'ostéoporose primaire ou de maladies osseuses rénales (4, 6-11). L'essai BoneTRAP® peut être employé également dans le suivi de l'efficacité des traitements d'anti-résorption (6,9,12-18). *In vitro*, l'activité de la TRACP 5b medium reflète le nombre d'ostéoclastes (15,19), et l'essai BoneTRAP® peut ainsi déterminer le nombre d'ostéoclastes dans les cultures d'ostéoclastes humains.