MouseTRAP™ (TRAcP 5b) ELISA

Solid phase immunofixed enzyme activity assay for the determination of osteoclast-derived tartrate-resistant acid phosphatase form 5b (TRACP 5b) in mouse serum

For Research Use Only. Not for use in diagnostic procedures

REF SB-TR103 Σ 96

Intended Use

For Research Use Only. Not for use in diagnostic procedures.

The MouseTRAP™ (TRAcP 5b) ELISA test is a solid phase immunofixed enzyme activity assay for the determination of mouse tartrate-resistant acid phosphatase form 5b (TRACP 5b).

Summary and Explanation

High amount of tartrate-resistant acid phosphatase (TRACP) is expressed by bone-resorbing osteoclasts and activated macrophages (1). Two forms of TRACP circulate in blood, known as TRACP 5a and TRACP 5b (2). TRACP 5b is derived from osteoclasts and TRACP 5a from inflammatory macrophages (3,4). 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 (5,6). Diurnal variability of serum TRACP 5b is low and the levels are not affected by feeding, allowing sample collection at any time of day (6). Recent studies have shown that secreted TRACP 5b indicates the number of osteoclasts rather than their activity (7-10).

The MouseTRAP™ (TRAcP 5b) ELISA assay is a specific method for the determination of TRACP 5b activity in mouse serum samples. Because the strain, sex and age of the animals used influences the values obtained, each laboratory should determine a reference range for the animals that are used. The MouseTRAP™ (TRAcP 5b) ELISA assay can also be used in in vitro mouse osteoclast cultures to measure TRACP 5b activity from cell lysates or culture medium. Because secreted TRACP 5b indicates the number of osteoclasts, TRACP 5b values determined from mouse osteoclast culture medium can be used to replace microscopic counting of the number of osteoclasts (7), and serum TRACP 5b values can be used to replace histological determination of osteoclast number in mouse bone.

Method Description

The MouseTRAP™(TRAcP 5b) ELISA assay uses a polyclonal antibody (7) prepared using recombinant mouse TRACP (11) as antigen. In the test, the antibody is incubated in anti-rabbit IgG-coated microtiter wells. After washing, standard, control, and samples are incubated in the wells, and bound TRACP 5b activity is determined with a chromogenic substrate to develop color. The reaction is stopped, and the absorbance of the reaction mixture is read in a microtiter plate reader, color intensity being directly proportional to the amount and activity of TRACP 5b present in the sample.

Warnings and Precautions

The MouseTRAP™ (TRAcP 5b) ELISA assay is *for research use only* and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. Immunodiagnostic Systems Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Sodium Azide

Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Calibrators CAL and Control CTRL

Classification under CLP:

Acute Tox. 4

Aquatic Chronic 3

EUH032

Hazard statements:

EUH032:	Contact with acids liberates very toxic gas.
H302:	Harmful if swallowed.
H412:	Harmful to aquatic life with long lasting effects.

Precautionary statements:

P264:	Wash hands thoroughly after handling.
P270:	Do not eat, drink or smoke when using this product.
P273:	Avoid release to the environment.
P301+310:	IF SWALLOWED: Immediately call a POISON CENTER or doctor.
P330:	Rinse mouth.
P501:	Dispose of contents/container to hazardous or special waste collection point.

Preparation of Reagents

Calibrators CAL and Control CTRL : Calibrators CAL and Control CTRL are supplied lyophilised. Reconstitute with 0.5 mL of distilled or deionised water, replace stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution.

If Calibrators and Control are to be used more than once, they must be frozen (-70°C recommended for a period longer than one week).

Anti-MouseTRAP Antibody: Anti-MouseTRAP Antibody Ab is supplied lyophilised. Reconstitute with 10.5 mL of distilled or deionised water, replace stopper and stand for 15 minutes at room temperature. Invert several times to ensure complete reconstitution.

Substrate Solution: Prepare just before use. Dissolve two Substrate Tablets SUBS pNPP in 10 mL of Substrate Buffer SUBSBUF (one tablet in 5 mL for half of the plate). If the substrate solution is to be used more than once, it must be stored frozen and protected from light.

Wash Buffer: Add the contents of each bottle of Wash Buffer Concentrate WASHBUF 25x to 960 mL of distilled or de ionised water and mix.

All other reagents are supplied ready for use.

Allow all reagents to come to room temperature before use. Reagents should be mixed by repeated inversion before use in the assay.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Specimen Collection and Storage

The assay should be performed using serum specimens. Specimens should be separated as soon as possible after collection. For long term storage, store at -80°C. Avoid repeated freeze/thaw of samples

Note:
The specimens' storage and stability information stated above are general recommendations for use in a variety of settings of laboratories. Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations to establish its own specimens handling and storage stability. For guidance on appropriate practices, please refer to the CLSI GP44-A4, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline - Fourth Edition

Procedure

Materials Provided

1. **CAL 0 - 4 - Calibrators (REF SB-TR103 01A - SB-TR103 01E):**
   Lyophilised Tris-buffered saline containing recombinant MouseTRAP and protein with 0.09% sodium azide. The exact value of each calibrator is printed on the QC Report. 0.5 mL per bottle, 5 bottles per kit.
2. **Ab - Anti-MouseTRAP Antibody (REF SB-TR103 02):**
   Lyophilised Tris-buffered saline containing anti-MouseTRAP antibody, protein, stabilisers and 0.05% sodium azide.
3. **MICROPLAT - Antibody Coated Plate (REF SB-TR103 02W):**
   Microplate with polyclonal anti-rabbit IgG linked to the inner surface of the polystyrene wells, 8 x 12-well strips in a pack with desiccant.
4. **CTRL - Control (REF SB-TR103 05)**:
   Lyophilised Tris-buffered saline containing recombinant MouseTRAP and protein with 0.09% sodium azide. The exact value of each control is printed on the QC Report. 0.5 mL per bottle.
5. **NaOH - Stop Solution (REF SB-TR000 06)**:
   0.32 M Sodium Hydroxide, 6 mL per bottle.
6. **RELEASREAG - Releasing Reagent (REF SB-TR103 07):**
   Proprietary reagent for dissociating TRAP from binding proteins. 4 mL per bottle.
7. **SUBS pNPP - Substrate Tablets (REF SB-TR000 08**):
   pNPP. 2 tablets.
8. **SUBSBUF - Substrate Buffer (REF SB-TR000 08B)**:
   Sodium Acetate buffer, 10 mL per bottle.
9. **WASHBUF 25x - Wash Buffer Concentrate (REF SB-TR000 09)**:
   Tris-buffered saline containing Tween-20, 40 mL per bottle.
10. **Adhesive Plate Sealer**.

Materials Required but not Provided

1. 0.9% NaCl.
2. Precision pipetting devices to deliver 20 µL, 25 µL, 50 µL ,100 µL, 500 µL and 5 mL.
3. Precision multi-channel pipettes to deliver 25 µL, 50 µL and 100 µL.
4. Automatic microplate washer (optional).
5. Photometric microplate reader and data analysis equipment

Assay Procedure

Bring all reagents to room temperature. Prepare the reagents as described in Preparation of Reagents. Transfer the required number of microtitre strips to a strip frame. Duplicate determinations are recommended. Return the remaining strips to the plastic tray pack and reseal. Do not take more strips than can easily be handled within 30 minutes. Note! Make sure that the plastic tray pack with desiccant remains sealed during storage.

1. Add **100 µL** of Anti-MouseTRAP Antibody to the appropriate wells of the Antibody Coated Plate MICROPLAT.
2. Incubate the plate at room temperature (20-24°C) for 60 minutes with shaking (approximately 950rpm).
3. Wash all wells four times with Wash Buffer:
   a) Automatic plate wash: Set plate washer to dispense at least 300 µL of Wash buffer per well.

Fill and aspirate for 4 cycles.

b) Manual wash: Decant the contents of the wells by inverting sharply. Dispense 250 µL of Wash Buffer to all wells. Decant and repeat three times.

Tap the inverted plate firmly on absorbent tissue to remove excess Wash Buffer before proceeding to the next step.

4. Add **100 µL** of each Calibrator CAL or Control CTRL to the appropriate wells of the Antibody Coated Plate MICROPLAT in duplicate.
5. Add **75 µL** of 0.9% NaCl followed by **25 µL** of sample to the appropriate wells of the Antibody Coated Plate MICROPLAT in duplicate.
6. Add **25 µL** of Releasing Reagent RELEASREAG to all wells using a multichannel pipette.
7. Incubate the plate at room temperature (20-24°C) for 60 minutes with shaking (approximately 950rpm).
8. Repeat Wash Step 3.
9. Add **100 µL** of freshly prepared Substrate solution to all wells using a multichannel pipette.
10. Cover the plate with an adhesive plate sealer. Incubate at 37°C for 2 hours.
11. Add **25 µL** of Stop Solution NaOH to all wells using a multichannel pipette. Mix the contents of the wells thoroughly.
12. Measure the absorbance of each well at 405 nm using a microplate reader within 30 minutes of adding the Stop Solution.

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. One kit control is provided. The control should be tested as an unknown. Quality Control charts should be maintained to follow the assay performance.

Calculation of Results

Plot the mean absorbance for each calibrator on the ordinate against concentration on the abscissa on log-lin graph. Read values for each control and unknown sample from the calibration curve in U/L. Multiply the results of the samples with dilution factor.

To obtain the concentration of MouseTRAP in each sample, multiply the value read from the curve by the dilution factor use**d (1:4)**

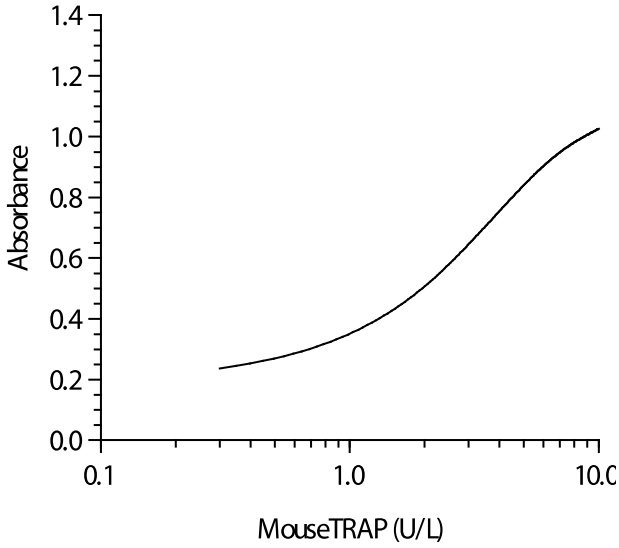
Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result. The exact value of each calibrator is printed on the QC Report

Well	Description	Abs.	Mean. Abs.	Result U/L
A1, A2	Calibrator 0 0 U/L	0.180 0.174	0.177	
A3, A4	Calibrator 1 0.3 U/L	0.240 0.234	0.237	
A5, A6	Calibrator 2 1.0 U/L	0.351 0.351	0.351	
A7, A8	Calibrator 3 3.0 U/L	0.649 0.640	0.645	
A9, A10	Calibrator 4 10.0 U/L	1.032 1.021	1.026	
A11, A12	Sample	0.424 0.436	0.430	1.5

Typical Calibration Curve

This sample calibration curve is for illustration only.



Performance Data

Sensitivity

The sensitivity is 0.1 U/L.

Precision

Intra-assay variation of the assay is <6.5%, and interassay variation is <8%

References

1 Yaziji H, Janckila AJ, Lear SC, Martin AW, Yam LT 1995 Immunohistochemical detection of tartrate-resistant acid phosphatase in non-hematopoietic human tissues. Am J Clin Pathol 104:397-402.

2 Lam KW, Li CY, Yam LT, Desnick RJ 1981 Comparison of the tartrate-resistant acid phosphatase in Gaucher's disease and leukemic reticuloendotheliosis. Clin Biochem 14:177-181.

3 Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Väänänen HK 2000 Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. J Bone Miner Res 15:1337-1345.

4 Janckila AJ, Neustadt DH, Nakasato YR, Halleen JM, Hentunen T, Yam LT 2002 Serum tartrate-resistant acid phosphatase isoforms in rheumatoid arthritis. Clin Chim Acta. 320:49-58.

5 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.

6 Hannon RA, Clowes JA, Eagleton AC, Al Hadari AA, Eastell R, Blumsohn A 2004 Clinical performance of immunoreactive tartrate resistant acid phosphatase isoform 5b as a marker of bone resorption. Bone 34:187-194.

7 Alatalo SL, Halleen JM, Hentunen TA, Mönkkönen J, Väänänen HK 2000 Rapid screening method for osteoclast differentiation in vitro that measures tartrate-resistant acid phosphatase 5b activity secreted into the culture medium. Clin Chem 46:1751-1754.

8 Alatalo SL, Penq Z, Janckila AJ, Kaija H, Vihko P, Väänänen HK, Halleen JM 2003 A novel immunoassay for the determination of tartrate-resistant acid phosphatase 5b from rat serum. J Bone Miner Res 18:134-139.

9 Chu P, Chao TY, Lin YF, Janckila AJ, Yam LT 2003 Correlation between histomorphometric parameters of bone resorption and serum type 5b tartrate-resistant acid phosphatase in uremic patients on maintenance hemodialysis. Am J Kidney Dis 41:1052-1059.

10 Alatalo SL, Ivaska KK, Waguespack SG, Econs MJ, Väänänen HK, Halleen JM 2004 Osteoclast-derived serum tartrate-resistant acid phosphatase 5b in Albers-Schonberg disease (type II autosomal dominant osteopetrosis). Clin Chem 50:883-890.

11 Marshall K, Nash K, Haussman G, Cassady I, Hume D, de Jersey J, Hamilton S (1997). Recombinant human and mouse purple acid phosphatases: expression and characterization. Arch Biochem Biophys 15:230-23

	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Use By* *Verwendbar bis* *Fecha de caducidad* *Utilizzare entro* *Utiliser jusque* *Houdbaar tot* *Holdbar til* *Použitelné do* *Použiteľné do* *Ημερομηνία λήξης* *Prazo de validade* *Felhasználható* *Använd före* *Użyć przed*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Batch code* *Chargenbezeichnung* *Código de lote* *Codice del lotto* *Code du lot* *Lot nummer* *Lotnummer* *Číslo šarže* *Číslo šarže* *Αριθμός Παρτίδας* *Código do lote* *Sarzsszám* *Lot nummer* *Kod partii*
	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Catalogue number* *Bestellnummer* *Número de catálogo* *Numero di catalogo* *Référence du catalogue* *Catalogus nummer* *Katalognummer* *Katalogové číslo* *Katalógové číslo* *Αριθμός καταλόγου* *Referência de catálogo* *Katalógusszám* *Katalognummer* *Numer katalogowy*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Manufacturer* *Hersteller* *Fabricante* *Fabbricante* *Fabricant* *Fabrikant* *Producent* *Výrobce* *Výrobca* *Κατασκευαστής* *Fabricante* *Gyártó* *Tillverkare* *Producent*
	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Contains sufficient for <n> tests* *Inhalt ausreichend für <n> Prüfungen* *Contenido suficiente para <n> ensayos* *Contenuto sufficiente per "n" saggi* *Contenu suffisant pour "n"tests* *Inhoud voldoende voor "n" testen* *Indeholder tilsttrækkeligt til "n" test* *Lze použít pro <n> testů* *Obsah postačuje na <n> stanovení* *Περιεχόμενο επαρκές για «ν» εξετάσεις* *Conteúdo suficiente para "n" ensaios* *A doboz tartalma <n> vizsgálat elvégzéséhez elegendő* *Räcker till "n" antal tester* *Wystarczy na wykonanie <n> testów*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*In Vitro Diagnostic Medical Device* *In-Vitro-Diagnostikum* *Producto sanitario para diagnóstico in vitro* *Dispositivo medico-diagnostico in vitro* *Dispositif médical de diagnostic in vitro* *Medisch hulpmiddel voor in-vitro diagnostiek* *Medicinsk udstyr til in vitro-diagnostik* *In Vitro diagnostický zdravotnický prostředek* *Zdravotnícka pomocka in vitro* *In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν* *Dispositivo médico para diagnóstico in vitro* *In vitro diagnosztikum* *Medicintekniska produkter för in vitro diagnostik* *Wyrób do diagnistyki In Vitro*
	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Temperature limitation* *Temperaturbegrenzung* *Límite de temperatura* *Limiti di temperatura* *Limites de température* *Temperatuurlimiet* *Temperaturbegrænsning* *Teplotní rozmezí od do* *Teplotné rozmedzie od do* *Περιορισμοί θερμοκρασίας* *Limites de temperatura* *Hőmérséklettartomány* *Temperaturbegränsning* *Przestrzegać zakresu temperatury*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Consult Instructions for Use* *Gebrauchsanweisung beachten* *Consulte las instrucciones de uso* *Consultare le istruzioni per l'uso* *Consulter les instructions d'utilisation* *Raadpleeg de gebruiksaanwijzing* *Se brugsanvisning* *Viz návod k použití* *Viď návod na pužitie* *Συμβουλευτείτε τις οδηγίες χρήσης* *Consulte as instruções de utilização* *Nézze meg a Használati utasítást* *Se handhavandebeskrivningen* *Sprawdź w instrukcji obsługi*

Procedure Summary

ASSAY

Add 100 µL Anti-MouseTRAP Antibody to Antibody Coated Plate MICROPLAT .

↓

Incubate: 60 minutes @ 20-24°C with shaking.

↓

Wash plate.

↓

Add 100 µL Calibrator CAL or Control CTRL to Antibody Coated Plate MICROPLAT.

Add 75 µL 0.9% NaCl followed by 25 µL sample to Antibody Coated Plate MICROPLAT

Add 25 µL Releasing Reagent RELEASREAG

↓

Incubate: 60 minutes @ 20-24°C with shaking.

↓

Wash plate.

↓

Add 100 µL Substrate solution.

↓

Incubate: 2 hours @ 37°C.

↓

Add 25 µL Stop Solution NaOH.

↓

Read plate @ 405nm.

Immunodiagnostic Systems Limited.

UK Immunodiagnostic Systems Limited, 10 Didcot Way, Boldon Business Park, Boldon, Tyne & Wear, NE35 9PD, UK
Tel: +44 (0) 191 519 0660 • Fax: +44 (0) 191 519 0760 • e-mail: info.uk@idsplc.com • www.idsplc.com
USA Immunodiagnostic Systems (IDS) Inc, 948 Clopper Road, Gaithersburg, MD 20878, USA
Tel: +1(877)852-6210 • Fax: +1 (301)990-4236 • e-mail: info.us@idsplc.com • www.idsplc.com
Germany Immunodiagnostic Systems GmbH (IDS GmbH), Mainzer Landstrasse 49, 60329 Frankfurt am Main
Tel: +49 (0) 69 3085-5025 • Fax: +49 (0) 69 3085-5125 • e-mail: info.de@idsplc.com • www.idsplc.com
France Immunodiagnostic Systems France SA (IDS France SA) 153 Avenue D'Italie, 75013 PARIS
Tel: +33 (0)1 40 77 04 50 • Fax: +33 (0)1 40 77 04 55 • e-mail: info.fr@idsplc.com • www.idsplc.com
Scandinavia Immunodiagnostic Systems Nordic a/s (IDS Nordic a/s), International House, Center Boulevard 5, 2300, København S, Denmark
Tel:+45 44 84 0091 • email: info.nordic@idsplc.com • www.idsplc.com