コスモ・バイオ株式会社

immunodiagnosticsystems

RatTRAP™ Assay

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

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

REF SB-TR102 Σ 96

Intended Use

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

The RatTRAP™ test is a solid phase immunofixed enzyme activity assay for the determination of rat tartrate-resistant acid phosphatase 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 RatTRAP™ assay is a specific method for the determination of TRACP 5b activity in rat serum samples (8). 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. Rat ovariectomy (OVX) and orchidectomy (ORX) models are the most commonly used experimental animal models of osteoporosis. In these models, TRACP 5b values should be measured before the operation and at 7, 14 and 28 days after the operation. In longer experiments, the measurements should be repeated monthly after day 28. Serum TRACP 5b values are expected to decrease after OVX and ORX because TRACP 5b describes the number of osteoclasts, and the histomorphometrically determined total number of osteoclasts in bone tissue (N.Oc/T.Ar) is decreased after OVX and ORX due to substantial bone loss caused by the operations (8,11). The RatTRAP™ assay can also be used in in vitro rat 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 rat 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 rat bone (8,11).

Method Description

The RatTRAP™ assay uses a highly characterized, speci□c monoclonal antibody prepared using baculovirus-generated recombinant rat TRACP as antigen (12). In the test, the monoclonal antibody is incubated in anti-mouse IgG-coated microtiter wells. After washing, standard, control, and serum 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 activity of TRACP 5b present in the sample.

Warnings and Precautions

The RatTRAP™ 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. IDS 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.

0.32M sodium hydroxide

Stop Solution NaOH contains 0.32M sodium hydroxide.
R36/38 Irritating to eyes and skin.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S37/39 Wear suitable gloves and eye/face protection.

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-RatTRAP Antibody: Anti-RatTRAP 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.

Procedure

Materials Provided

1. **CAL 1-4 - Calibrators (REF SB-TR102 01A - SB-TR102 01D):**

   Lyophilised Tris-buffered saline containing recombinant RatTRAP and protein with 0.09% sodium azide. The exact value of each calibrator is printed on the bottle label. 0.5 mL per bottle, 4 bottles per kit.

2. **Ab - Anti-RatTRAP Antibody (REF SB-TR102 02):**

   Lyophilised Tris-buffered saline containing anti-RatTRAP antibody, protein, stabilisers and 0.05% sodium azide.

3. **MICROPLAT - Antibody Coated Plate (REF SB-TR102 02W):**

   Microplate with polyclonal anti-mouse 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-TR102 05)**:

   Lyophilised Tris-buffered saline containing recombinant RatTRAP and protein with 0.09% sodium azide. The exact value of each control is printed on the bottle label. 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-TR102 07):**

   Proprietary reagent for dissociating TRAP from binding proteins. 6 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.

Open the foil of the plastic tray pack of the plate from three sides only and fold aside leaving the plate-specific information on the package. Transfer the required number of microtitre strips to a strip frame. Return the remaining strips into the package and press the foil cover back on as tightly as possible. Leave the desiccant in the package. Store the package in the resealable plastic bag. 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-RatTRAP Antibody [Ab] 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. 0.9% NaCl (saline) is used for blank.
5. Add **25 µL** of sample and **75 µL** of 0.9% NaCl to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.
6. Add **50 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 60 minutes.
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 semi-log graph paper. 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 RatTRAP in each sample, multiply the value read from the curve by the dilution factor used (1:4). If the sample volume is 20 µL and volume of 0.9% NaCl is 80 µL, dilution factor is 5.

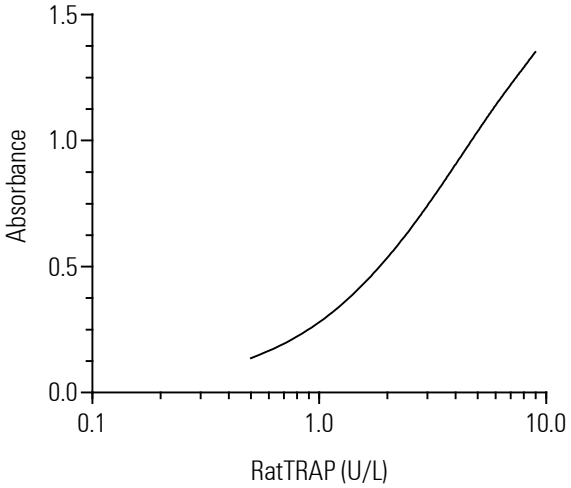
Sample Assay Data

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 bottle label.

Well	Description	Abs.	Mean. Abs.	Mean Abs - Blank	Result U/L
A1, A2	Blank	0.190 0.189	0.190	-	
A3, A4	Calibrator 1 0.5 U/L	0.326 0.327	0.326	0.137	
A5, A6	Calibrator 2 1.5 U/L	0.602 0.606	0.604	0.414	
A7, A8	Calibrator 3 3.0 U/L	0.928 0.935	0.931	0.742	
A9, A10	Calibrator 4 9.0 U/L	1.515 1.572	1.543	1.354	
A11, A12	Sample	0.703 0.692	0.697	0.508	1.9

Typical Calibration Curve

This sample calibration curve is for illustration only.



Performance Data

Precision

	Sample	Number of replicates	Mean value U/L	CV%
Intra-assay (within run) precision of the RatTRAP Assay	1	10	1.19	5.8
	2	10	2.42	3.5
	3	10	2.72	4.1
	4	10	6.15	5.2
	5	10	10.32	3.9
Inter-assay precision (between run) of the RatTRAP Assay	1	8	1.24	5.2
	2	8	2.42	3.2
	3	8	2.70	3.0
	4	8	5.99	3.9

Recovery

Spiked rat serum were prepared by adding varying levels of RatTRAP to pooled rat serum specimen containing a known amount of RatTRAP. The mean recovery was 99% (range 92-105%).

Sensitivity

The sensitivity of the assay is defined as two standard deviations above the mean optical density of 16 BLANK replicates. The corresponding concentration was calculated as 0.1 U/L.

References

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Issue: 5
Date: 31 March 2009

Procedure Summary

ASSAY

Add 100 µL Anti-RatTRAP Antibody **Ab** to Antibody Coated Plate **MICROPLAT**.

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

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Wash plate.

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Add 100 µL Calibrator **CAL** or Control **CTRL** (0.9% NaCl for blank) to Antibody Coated Plate **MICROPLAT**.

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

Add 50 µL Releasing Reagent **RELEASREAG**

↓

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

↓

Wash plate.

↓

Add 100 µL Substrate solution.

↓

Incubate: 60 minutes @ 37°C.

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Add 25 µL Stop Solution **NaOH**.

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Read plate @ 405nm.

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