

## MouseTRAP™ Assay

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.*

## Intended Use

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

The MouseTRAP™ 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™ 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™ 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™ 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™ 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-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.

## 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 bottle label. 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 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-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 [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.
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 used (1:4).**

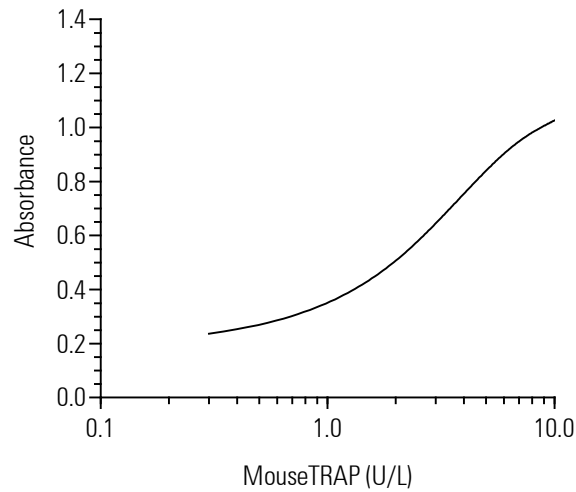
## Sample Assay Data

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 bottle label.

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









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## Procedure Summary

### ASSAY

Add 100 µL Anti-MouseTRAP Antibody **Ab** 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.



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