RatTRAP™ (TRAcP 5b) ELISA

For Research Use Only Instructions For Use

Product Name	RatTRAP™ (TRAcP 5b)	REF	SB-TR102
Abbreviated Product Name	RatTRAcP		

1. Intended Use

Not for use in clinical or diagnostic procedures.

The RatTRAP™ (TRAcP 5b) ELISA is intended for the quantitative determination of rat tartrate-resistant acid phosphatase 5b (TRAcP 5b) in rat serum. Results are to be used for research use only.

2. 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].
text added if required

3. Method Description

The RatTRAP™ (TRAcP 5b) ELISA uses a highly characterised, specific 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, either 25 µL of patient sample plus 75 µL of diluent or 100 µL of each calibrator or control are incubated in the wells. Following a further wash step, the bound TRAcP 5b activity is determined with a chromogenic substrate to develop colour. The reaction is stopped and the absorbance of the reaction mixture is read in a microtiter plate reader, colour intensity being directly proportional to the activity of TRAcP 5b present in the original sample.

4. Warnings and Precautions

The RatTRAP™ (TRAcP 5b) ELISA 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 these Instructions For Use (IFU). Immunodiagnostic Systems Limited (IDS) 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 practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Reagents containing Sodium Azide
Some reagents in this kit contain sodium azide (NaN_3) <0.1 % (w/w) 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.

Classification according to Regulation (EC) CLP:
Acute toxicity (oral), Category 4
Hazardous to the aquatic environment – Chronic Hazard, Category 3

Warning

Contains Sodium Azide

Hazard statements:
H302 - Harmful if swallowed.
H412 - Harmful to aquatic life with long lasting effects.

Precautionary statements
P264 - Wash hands, forearms and face thoroughly after handling.

5. Shelf Life And Storage Of Reagents

Store the assay kit in an **upright** position at 2 - 8 °C. **Do not freeze**.
This kit is stable until the stated expiry date if stored as specified. Do not use any kit component beyond their expiry date.

Reagent	**After opening or preparation**
Substrate Solution	Store at ≤ -20° protected from light after preparation
Calibrators	Store at ≤ -70° protected for period > than 1 week after preparation
Controls	Store at ≤ -70° protected for period > than 1 week after preparation

6. Sample Collection and Storage

The assay should be performed using serum samples. Samples should be separated as soon as possible following collection. For long term storage, store at -80°C.

Note:

- Samples containing particulate matter must be centrifuged before performing the assay. Centrifuged samples with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified samples without the lipemic material.
- Samples displaying microbial contamination, highly lipemic or grossly haemolysed should not be assayed with the kit.
- Before performing assays, make sure that samples, calibrators and controls are at room temperature (20 - 24 °C).
- Do not use heat-inactivated samples.
- Avoid repeat freeze/thaw cycles for samples.
- 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".

7. Materials

Materials Provided

MICROPLAT **Antibody coated plate** **REF SB-TR102 02W**
Microwell strips (8x12 wells) pre-coated with polyclonal anti- mouse IgG, supplied in a plastic frame

AB **Anti- RatTRAP antibody** **REF SB-TR102 02**
Lyophilised anti- RatTRAP antibody in TRIS-buffered saline containing protein, stabilisers and sodium azide as preservative (0.05 %); 1 vial, 1.0 mL

RELEASREAG **Releasing reagent** **REF SB-TR102 07**
Proprietary reagent for dissociating TRAcP from binding proteins; 1 vial, 6.0 mL

SUBS pNPP **Substrate tablets** **REF SB-TR000 08**
pNNP tablets; 2 tablets

WASHBUF 25x **Washing Buffer** **REF SB-TR000 09**
Concentrated washing buffer with detergent and preservative; 1 vial, 40.0 mL

SUBSBUF **Substrate buffer** **REF SB-TR000 08B**
Sodium acetate buffer; 1 vial, 10.0 mL

NaOH **Stop Solution** **REF SB-TR000 06**
Ready to use solution of 0.32 M sodium hydroxide; 1 vial, 6.0 mL

CAL 1 – 4 **Calibrator** **REF SB-TR102 01A – SB-TR102 01D**
Lyophilised solution containing TRIS buffer containing recombinant RatTRAP and proteins with 0.09 % sodium azide as preservative; 1 each of 4 concentration levels, 0.5 mL per vial (reconstituted volume)
The exact value of each calibrator is printed on the QC report.

CTRL **Control** **REF SB-TR102 05**
Lyophilised solution containing TRIS buffer containing recombinant RatTRAP and proteins with 0.09 % sodium azide as preservative; 1 vial, 0.5 mL per vial (reconstituted volume)
The established range for the control is printed on the QC report.

Adhesive Plate Seals

Documentation Instructions for Use and QC report.

Materials Required But Not Provided
- 0.9% NaCl
- Containers for preparing the Antibody reagent and the Washing solution
- Precision pipetting devices to deliver 20 - 5000 µL
- Distilled or deionised water
- Precision 8 or 12 channel multipipette to deliver 25 µL, 50 µL and 100 µL
- Automatic microplate washer (optional)
- Photometric microplate reader and data analysis equipment

8. Preparation Of Reagents
Allow all reagents to come to room temperature for a minimum of 60 minutes before use. Do not interchange kit components from different lots.

Anti- RatTRAP antibody
Anti- RatTRAP antibody is supplied lyophilised. Reconstitute with 10.5 mL of distilled or deionised water, replace stopper and stand for 10 – 15 minutes at room temperature. Invert several times to ensure complete reconstitution.

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 the plate). Invert several times to ensure complete mixing.

Wash buffer
Add the contents of each bottle of Wash Buffer Concentrate WASHBUF 25x to 960 mL of distilled or deionised water and mix

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.

All other reagents are supplied ready for use and should be mixed by repeated inversion before use.

N.B. To avoid potential microbial and / or chemical contamination, unused reagents should never be returned into the original vials.

9. Assay Procedure
Prepare reagents as described in § 8. Preparation of Reagents. Mix all reagents and samples before use (avoid formation of foam).
NOTE: To ensure consistent results between runs, between operators, and to minimise any drift effect; strictly adhere to the following procedure:
a. Bring all reagents to room temperature (20 – 24 ºC) prior to use – this will take approximately 60 minutes.
b. Seal the plate during incubations using the plate sealers which are supplied with the assay kit.
c. Do not stack plates during incubation in order to ensure a consistent temperature for all plates.
d. Do not under or over-fill the assay wells during the washing steps.
e. Add reagents in the same sequence each time to reduce time deviation between reactions.

Determine the number of strips needed for the assay; it is recommended to test all samples in duplicate. In addition, for each run a total of 10 wells are needed for the standards and controls. Place the appropriate number of strips in the plastic frame. Store any unused strips in the tightly closed foil bag with desiccant capsules.

1. Pipette 100 µL of anti-RatTRAP Antibody AB to the appropriate wells of the Antibody Coated Plate MICROPLAT
2. Cover the plate with plate sealer and incubate the plate at room temperature (20 – 24 ºC) for 60 minutes with shaking (approx. 950 rpm)
3. Wash all wells 4 times with Wash Buffer

   Automatic plate wash — Set plate washer to dispense 300 µL of wash solution per well
   Fill and aspirate for 4 cycles

   Manual wash — Decant the contents of the wells by inverting sharply
   Pipette 250 µL of wash solution into each well, decant and repeat 3 times
   Remove excess wash buffer by tapping firmly on absorbent tissue before proceeding
4. Add 100 µL each calibrator CAL, control CTRL to the appropriately 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. Pipette 50 µL of Releasing Reagent RELEASREAG to all wells using a multichannel pipette.
7. Cover the plate with plate sealer and incubate the plate at room temperature (20 – 24 °C) for 60 minutes with shaking (approx. 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.

N.B. Microplate readers measure vertically; when pipetting, do not touch the bottom of the wells

Automated Platforms
The RatTRAP™ (TRAcP 5b) ELISA kit was designed and developed to be performed manually using the protocol described above. The protocol is not necessarily applicable to automated platforms.
If automated platforms are used it is the responsibility of the user to ensure the kit has been appropriately tested. To improve the performance of the kit on automated platforms, it is recommended to increase the number of wash cycles at each wash step.

10. 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

11. Quality Control

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

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.

IDS recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[13].

12. Symbols used

REF	Catalogue Number
	Manufacturer

13. Bibliography

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Assay Procedure