# MouseTRAP™ (TRAcP 5b) ELISA

For Research Use Only  Instructions For Use

| | | | |
|---|---|---|---|
| **Product Name** | MouseTRAP™ (TRAcP 5b) ELISA | REF | SB-TR103 |
| **Abbreviated Product Name** | MouseTRAcP | | |

## 1. Intended Use

**Not for use in clinical or 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).

## 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 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 has previously been used in *in vitro* mouse osteoclast cultures to measure TRAcP 5b activity from cell lysates or culture medium (not verified in house). 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.

## 3. Method Description

The MouseTRAP™ (TRAcP 5b) ELISA is an immunofixed enzyme activity assay where 25 µL of test sample is added to the microtitre plate followed by 75 µL of provided diluent, or 100 µL of calibrators or controls are added to the microtitre plate. The calibrators, controls or diluted test samples are incubated together with a releasing reagent in microtitre wells which are coated with polyclonal antibody[7] against recombinant mouse TRAcP[11]. Following the incubation, the plate is washed and a chromogenic substrate added. Following a further incubation step, the reaction is stopped; the absorbance of the reaction mixtures are read in a microtiter plate reader, colour intensity developed being directly proportional to the activity of TRAcP 5b in the original sample.

## 4. Warnings and Precautions

The MouseTRAP™ (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:**
Skin sensitisation, Category 1

Warning

Contains ProClin 300

Hazard statements:
H317 - May cause an allergic skin reaction.

Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P321 - Specific treatment (see supplemental first aid instruction on this label).

Example Version

P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.

## 5. Shelf Life And Storage Of Reagents

This kit is stable until the expiry date printed on the box if stored as specified. Upon receipt, store all reagents at 2-8°C. Do not use any kit component beyond their expiry date.

Indications of possible deterioration of kit reagents include:

- The presence of abnormal particulate matter in any of the reagents.
- A decrease in the maximum binding.
- A high non-specific binding.
- A shift in the slope of the curve from its normal position.

| **Reagent** | **After opening or preparation** |
|---|---|
| Calibrator / Controls | Stable up to 1 hr at 2 - 8°C or 30 mins at 22°C<br>Stable for 1 freeze/thaw cycle<br>Stable for 8 weeks if stored at -80°C after preparation |
| Test samples (based on limited in house testing) | Stable up to 2 hrs at 2 - 8°C or 1 hr at 22°C<br>Stable for 3 freeze/thaw cycles<br>For long-term, store at -80°C after preparation |

## 6. Sample Collection and Storage

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

**Note:**

- Some commercially available sample collection tubes may affect the results of testing in particular cases.
- Follow the blood collection tube manufacturer's recommendations for handling and processing the samples.
- 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 (18 - 22 °C).
- Do not use heat-inactivated samples.
- Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organisations 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**
Microwell strips (12x8 wells) pre-coated with polyclonal anti-Mouse TRAcP antibody, supplied in a plastic frame

**SAMPDIL** **Sample diluent**
Saline solution containing Proclin 300 as preservative (<0.05 %); 1 vial, 10.0 mL

**RELEASREAG** **Releasing Reagent**
Proprietary reagent for dissociating TRAcP from binding proteins; 1 vial, 4.0 mL. contains 0.1% Germall™ II as preservative.

**SUBS pNPP** **Substrate Tablets**
pNPP; 4 tablets

**SUBSBUF** **Substrate Buffer**
Sodium acetate buffer; containing 0.1% Germall™ II as preservative; 2 vials, 10.0 mL each

**WASHBUF 25x** **Washing Buffer**
Concentrated TRIS buffered saline washing buffer with Tween-20 detergent and 2.5% Germall™ II as preservative; 1 vial, 40 mL

Example Version

**NaOH** **Stop Solution**
Ready to use solution of 0.2 M Sodium Hydroxide; 1 vial, 14.0 mL

**CAL 0 – 4** Calibrators
Lyophilised TRIS buffered saline containing recombinant mouse-TRAcP with proteins and 0.09 % sodium azide as preservative; 1 each of 5 concentration levels, 0.5 mL per vial (reconstituted volume).
The exact value for each calibrator is printed on the QC report.

**CTRL** Control
Lyophilised TRIS buffered saline containing recombinant mouse-TRAcP with proteins and 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 Sealer** 4 per kit.

**Documentation** Instructions for Use and QC report.

**Materials Required But Not Provided**
- Container for preparing the Washing Solution
- Precision pipetting devices to deliver 25 to 5000µL
- Deionised water
- Precision 8 or 12 channel multipipette to deliver 25 µL, 75µL,100 µL and 300 µL
- Vortex mixer
- Microwell mixing apparatus
- 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.

**Calibrators CAL and Control CTRL:**
Calibrators **CAL** and Control **CTRL** are supplied lyophilised. Reconstitute with 0.5 mL of deionised water, replace stopper and stand for 10 – 15 minutes at room temperature (18 - 24°C). Invert several times to ensure complete reconstitution.

**Substrate Solution**
Prepare the Mouse TRAcP substrate solution approximately 15 mins prior to the end of step 4.
1 Substrate Tablet **SUBS pNPP** is dissolved in 5 ml Substrate Buffer **SUBSBUF**;

**Wash Solution**
Prepare the Wash Solution by mixing 1-part Washing Buffer **WASHBUF 25X** to 25-parts deionised water in an empty container. Mix carefully and avoid formation of foam.

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.

Example Version

## 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 kit reagents to room temperature (18 – 24 ºC) prior to use – this will take approximately 60 minutes.
b. It is recommended to add the samples to the plate within 20 minutes.
c. Seal the plate during incubations using the plate sealers which are supplied with the assay kit.
d. Do not stack plates during incubation in order to ensure a consistent temperature for all plates.
e. Do not under or over-fill the assay wells during the washing steps.
f. Add substrate within immediately following the washing step, do not let plates dry; stagger stop times as needed.
g. Add reagents in the same sequence each time to reduce time deviation between reactions

1. 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 12 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. Pipette 100 µL of each standard **CAL 0 - 4** and control **CTRL** to the appropriate wells on the Antibody Coated Plate **MICROPLAT** in duplicate
2. Pipette 25 µL of each unknown sample followed by 75 µL of Sample Diluent **SAMPDIL** to the appropriate wells on the Antibody Coated Plate **MICROPLAT** in duplicate
3. Pipette 25 µL of releasing agent **RELEASREAG** into each well
4. Incubate at room temperature (18 – 24 °C) 60 minutes on a microtiter plate mixer (900 rpm)

5. Approximately 15 minutes prior to the end of the incubation step, prepare the substrate solution according to § 8
6. Wash all wells 4 times with prepared wash solution **WASHBUF SOLN**
   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 300 µL of wash solution into each well, decant and repeat 4 times
   Make sure wells are completely emptied after each manual or automatic washing cycle.
   Remove excess wash buffer by tapping firmly on absorbent tissue before proceeding.
   Proceed immediately to the next step at the end of washing.
7. Pipette 100 µL of freshly prepared Substrate Solution into each well
8. Cover the plate with an adhesive plate seal and incubate at 37°C for 2 hours
9. Pipette 100 µL of Stopping Solution NaOH into each well
10. Measure absorbance at 405 nm with reference at 650 nm using a microplate reader within 30 minutes of adding the Stopping Solution

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

**Automated Platforms**
The MouseTRAcP 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 may be necessary to increase the number of wash cycles at each wash step.

## 10. Calculation of Results

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-PL fit, **including Calibrator 0 is required.**

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

**NOTE:** If the absorbance of a sample exceeds that of **Standard 4**, the sample should be diluted in **Sample Diluent SAMPDIL** and re-analysed.

## 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 kit control provided in the kit should be tested as unknown and is intended to assist in assessing the validity of results obtained with each assay plate.
The mean concentration of the control level is documented in the QC report included with each kit. The mean concentration level is determined over several assays run in duplicate. The established control range is equivalent to +/- 2 standard deviations based on a nominal % coefficient variation of the kit control.

IDS recommends the user to maintain graphic records of the control value 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[12].

## 12. Measurement Range (Reportable Range)

The measuring range of the assay is from 0.1 U/L. Any value that reads below 0.1 U/L should be reported as "< 0.1 U/L".

Samples with MouseTRAcP concentrations above the absorbance of calibrator 4 should be further diluted with Sample Diluent **SAMPDIL**. The results for these diluted samples must be multiplied by the corresponding overall dilution factor.

## 13. Traceability

The calibrators of this kit are standardised against in-house reference standards (purified MouseTRAcP in buffer).

Example Version

## 14. Performance Data

Representative performance data are shown for information only. Results obtained at individual laboratories may vary.

### 14.1 Precision

Precision was evaluated in accordance with a modified protocol based on CLSI EP-5A2, "Evaluation of Precision Performance of Quantitative Measurement Methods" using 1 lot of assay reagents.
Intra assay precision was assessed on a total of 5 serum run in 10 replicates in a single assay; inter assay precision was assessed on 5 serum samples run in duplicate in at least 20 assays with 2 operators and assessed across at least 5 days.

| Sample 1 | Intra assay | | | | Inter assay | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean Conc. (U/L) | SD | %CV | n | Mean Conc. (U/L) | SD | %CV |
| 1 | 10 | 3.03 | 0.10 | 3.3% | 20 | 3.03 | 0.22 | 7.2% |
| 2 | 9 | 7.17 | 0.40 | 5.6% | 20 | 3.04 | 0.16 | 5.2% |
| 3 | 10 | 3.74 | 0.20 | 5.2% | 20 | 3.88 | 0.27 | 7.0% |
| 4 | 10 | 1.73 | 0.12 | 6.9% | 20 | 1.89 | 0.12 | 6.1% |
| 5 | 10 | 1.23 | 0.06 | 5.1% | 20 | 1.35 | 0.09 | 6.9% |

### 14.2 Method Comparison

The new version of the MouseTRAcP assay was compared against the previous version of the kit, following CLSI EP-9A2, "Method Comparison and Bias Estimation Using Patient Samples". A total of 50 samples, selected to represent a wide range of MouseTRAcP concentrations [0.6 – 5.8 U/L], was assayed by each method. Passing Bablok regression analysis was performed on the comparative data:

| N | Slope | 95% CI | Intercept (U/L) | 95% CI | Coefficient of determination ($R^2$) |
|---|---|---|---|---|---|
| 50 | 1.00 | 0.90 to 1.14 | -0.20 | -0.64 to 0.03 | 0.88 |

Example Version

## 15. Symbols used

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|---|---|
| REF | Catalogue Number |
|  | Manufacturer |

## 16. Bibliography


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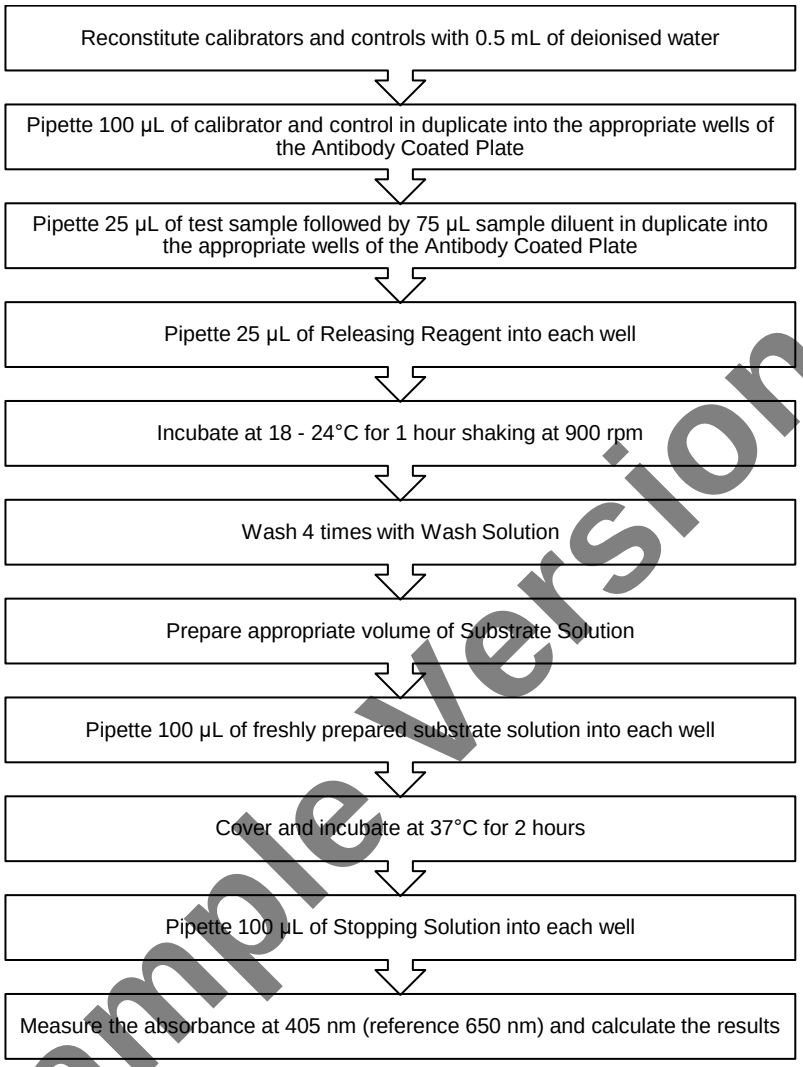
## Assay Procedure




Example Version