Manufactured and Distributed by:
Novatein Biosciences
710 Concord Ave, Cambridge, MA, 02138, USA
Phone: (617) 238-1396
Fax: (617) 380-0053
Toll Free: (888) 856-2858
http://www.novateinbio.com/
Email: Info@novateinbio.com

NOVATEINBIO
Accelerates Research and Development

Rat Thioredoxin,Trx ELISA

Catalog number: NB-E30134 (96 wells)

The kit is designed to detect the level of Rat Trx in cell culture supernatant, serum, plasma and other suitable sample solution

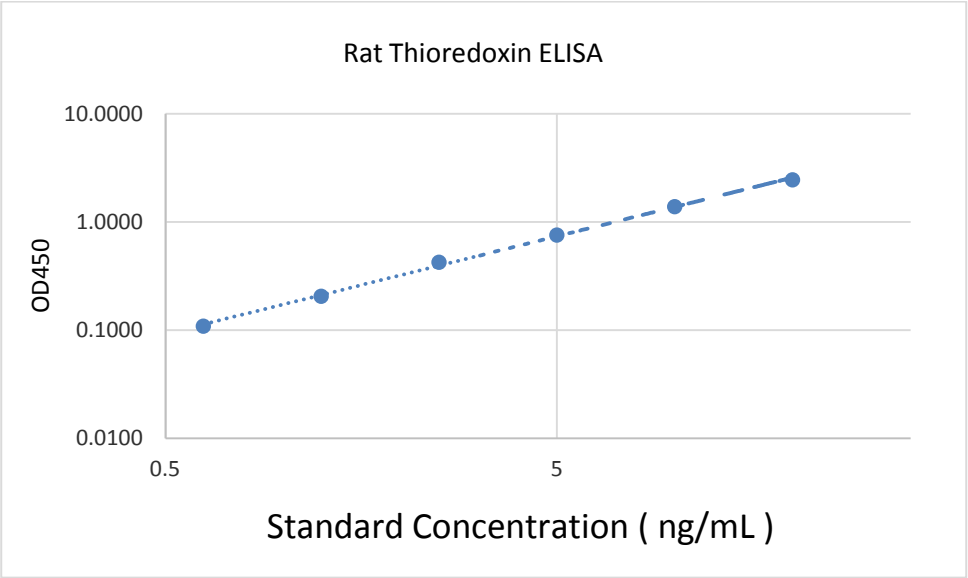
FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Result calculation

- This standard curve is used to determine the amount of an unknown sample. Construct a standard curve by plotting the average O.D. (450 nm) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve through the points on the graph.
- First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation. Construct the standard curve using graph paper or statistical software.
- To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
- Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

Typical data:

This standard curve was generated at Novateinbio for demonstration purpose only. A standard curve must be run with each assay.



3 Add 100µl of Conjugate to each well. Mix well. **Mixing well in this step is important.** Cover and incubate the plate for 1 hour at 37°C.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

   Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

   Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µl/well/wash) using an auto washer. After washing, dry the plate as above.It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

5. Add 50µl Chromogen Solution A and 50µl Chromogen Solution B to each well, subsequently. Cover and incubate for 15 minutes at 37°C. (Protect from light).
6. Add 50µl Stop Solution to each well. Mix well.
7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Important notes

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid from bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommended that all standard, testing samples are tested in duplicate.
- Use serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too shallow after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over develop)
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.
- Chromogen Solution B is light-sensitive. Avoid prolonged exposure to the light.

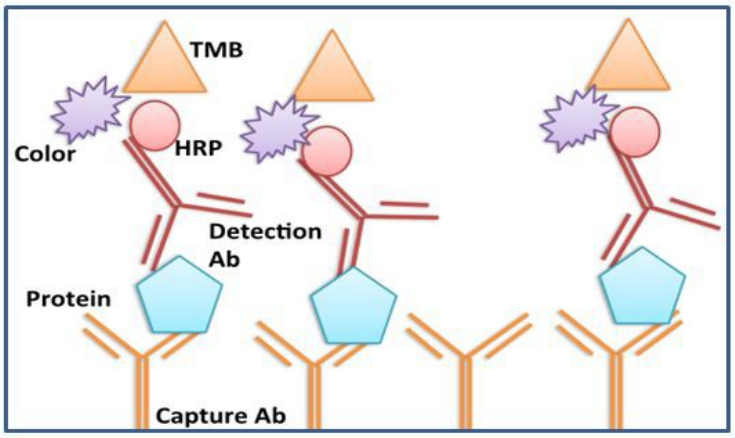
Intended use

The kit is used to quantify the Rat Trx in cell culture supernatant, serum, plasma and other suitable sample solution. This assay has high sensitivity and excellent specificity for detection of Rat Trx. No significant cross-reactivity or interference between Trx and analogues wasobserved.

Standard range	0.625- 20ng/ml
Sensitivity	0.1 ng/ml
Assay time	90 min
Validity	Six months
Store at	2-8 °C

Assay principle

This Rat Trx enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for Rat Trx. Standards or samples are then added to the microtiter plate wells and Trx if presents, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of Trx present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated antibody, specific for Trx are added to each well to “sandwich” the Trx immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain Trx and enzyme-conjugated antibody will exhibit a change in colour. The enzyme- substrate reaction is terminated by addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.



Materials supplied

1	Microelisa Stripplate	96 well
2	Standard	0.5 ml X 6 vials
3	20 X Wash Solution	25 ml
4	Sample Diluent	6 ml
5	HRP-Conjugate Reagent	10 ml
6	Chromogen Solution A	6 ml
7	Chromogen Solution B	6 ml
8	Stop Solution	6 ml
9	Closure plate membrane	2
10	Package insert	1

Note: Standard (S1 - S6) concentration was followed by: 0.63, 1.25, 2.5, 5, 10, 20 ng/ml

Materials required but not supplied

- 37℃ incubator.
- ☐ Standard plate reader capable of measuring absorbance at 450 nm.
- ☐ Adjustable pipettes and disposable pipette tips.
- ☐ Distilled water.
- ☐ Multi-channel pipettes, manifold dispenser or automated microplate washer.
- ☐ Absorbent paper.
- ☐ Materials used for sample preparation.

Sample collection and storage

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.
- **Cell culture fluid and other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

- Serum, plasma, and cell culture fluid samples to be used within 3 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤1months) or -80°C (≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.
- ☐ Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. P lease reserve sufficient amount of samples in advance.

- ☐ Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their part icular experiments.
- ☐ If the samples are not indicated in the manual, a preliminary experiment to determine the vali dity of the kit is necessary.
- ☐ Owing to the possibility of mismatching between antigen from other resource and antibody u sed in our kits (e.g., antibody targets conformational epitope rather than linear epitope), som e native or recombinant proteins from other manufacturers may not be recognized by our pro ducts.
- ☐ Influenced by the factors including cell viability, cell number and also sampling time, sam ples from cell culture supernatant may not be detected by the kit.
- Can't detect the samples containing NaN3, since NaN3 inhibits HRP (horseradish peroxidase) activity.
- ☐ Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.
- □ Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- Wash Solution-Dilute 25mL of Wash Solution concentrate(20 ×)with 475mL of deionized or distilled water to prepare 500 mL of Wash Solution (1 ×).

Assay procedures

1. Prepare all the Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples should be added in duplicate to the Microtiter Plate.
2. a) Assign standard wells, sample wells on the assay plate/strip.
   b) Add Sample Diluent only at 50 ul/well to 2 Standard wells (for duplicate) serving as the zero standard; take the Standards and agitate gently then add the pre-diluted standard at 50 µl/well to other standard wells following the sequence of S1 to S6.
   c) Add sample at 50µl/well to sample wells.

Note: If samples generate values higher than the highest standard, please further dilute the samples with the Sample Diluent and repeat the assay.