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User's Manual

Trf (Mouse) ELISA Kit

REF **DEIA4398**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

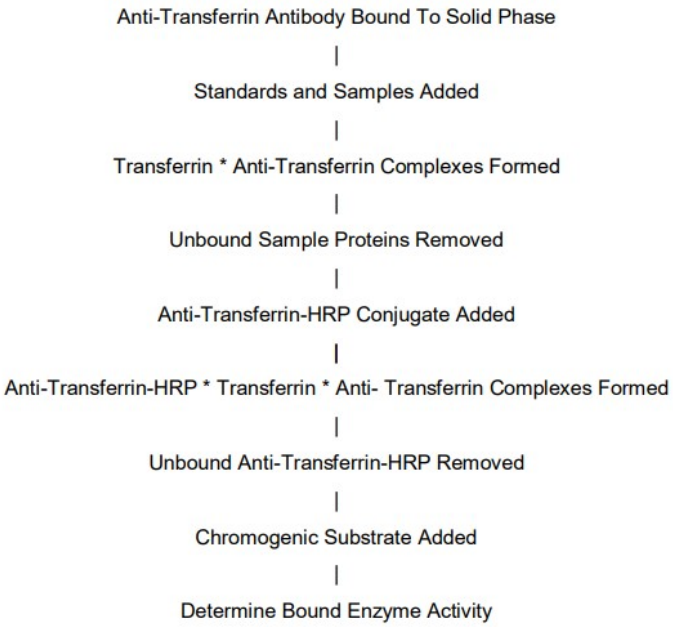
The Trf (Mouse) ELISA Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Transferrin in biological samples of mice.

General Description

Transferrin is a metal-combining protein that is reversibly bound to acid-soluble iron in plasma. It functions to transport iron to the bone marrow and to tissue storage organs such as the liver. Transferrin also participates in the regulation and control of iron absorption and protects against iron intoxication. Like haptoglobin, the carrier of hemoglobin, transferrin is synthesized in the liver, but unlike haptoglobin, transferrin is returned to the circulation after unloading its iron in the reticuloendothelial system. This ELISA kit can be used to measure transferrin in serum, tissue extracts and other biological fluids.

Principles of Testing

The principle of the double antibody sandwich ELISA is represented in following figure. In this assay the Transferrin present in samples reacts with the anti-Transferrin antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-Transferrin antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound Transferrin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'- tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of Transferrin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Transferrin in the test sample. The quantity of Transferrin in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.



Reagents And Materials Provided

1. Diluent Concentrate (Running Buffer): One bottle containing a 5× concentrated diluent running buffer. 50 mL
2. Wash Solution Concentrate: One bottle containing a 20× concentrated wash solution. 50 mL
3. Enzyme-Antibody Conjugate (100×): One vial containing affinity-purified anti-Mouse Transferrin antibody conjugated with horseradish peroxidase in a stabilizing buffer. 150 µL
4. Chromogen-Substrate Solution: One vial containing 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3. 12 mL
5. Stop Solution: One vial containing 0.3 M sulfuric acid. WARNING: Avoid contact with skin. 12 mL
6. Anti-Mouse Transferrin ELISA Micro Plate: Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity-purified anti-Mouse Transferrin. 96 (8x12) wells
7. Mouse Transferrin Calibrator: One vial containing a lyophilized Mouse Transferrin calibrator. 1 vial

Materials Required But Not Supplied

1. Precision pipettes (2 µL to 200 µL) for making and dispensing dilutions
2. Test tubes
3. Microplate washer/aspirator
4. Distilled or deionized H_2O
5. Microtitre Plate reader
6. Assorted glassware for the preparation of reagents and buffer solutions
7. Timer

Storage

The expiration date for the package is stated on the box label.

1. Diluent: The 5× Diluent Concentrate is stable until the expiration date. The 1× working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.
2. Wash Solution: The 20× Wash Solution Concentrate is stable until the expiration date. The 1× working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.
3. Enzyme-Antibody Conjugate: Undiluted horseradish peroxidase anti-Transferrin conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.
4. Chromogen-Substrate Solution: The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.
5. Stop Solution: The Stop Solution should be stored at 4-8°C and is stable until the expiration date.
6. Anti-Mouse Transferrin ELISA Micro Plate: Anti-Mouse Transferrin coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.
7. Mouse Transferrin Calibrator: The lyophilized Mouse Transferrin calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (avoid multiple

freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

Specimen Collection And Preparation

Specimen Collection and Handling

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation, by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Dilution of Samples

The assay for quantification of Transferrin in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/100,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended. To prepare a 1/100,000 dilution of sample, transfer 5 µL of sample to 1,995 µL of 1× diluent. This gives you a 1/400 dilution. Next, dilute the 1/400 samples by transferring 4 µL, to 996 µL of 1× diluent. You now have a 1/100,000 dilution of your sample. Mix thoroughly at each stage.

Reagent Preparation

1. Diluent Concentrate: The Diluent Solution supplied is a 5× Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O).
2. Wash Solution Concentrate: The Wash Solution supplied is a 20× concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.
3. Enzyme-Antibody Conjugate: Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1× Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.
4. Chromogen-Substrate Solution: Ready to use as supplied.
5. Stop Solution: Ready to use as supplied.
6. Anti-Mouse Transferrin ELISA Micro Plate: Ready to use as supplied. Unseal microtiter pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
7. Mouse Transferrin Calibrator: Add 1.0 mL of distilled or de-ionized water to the Mouse Transferrin calibrator and mix gently until dissolved. The calibrator is now at a concentration of 7.45 µg/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Mouse Transferrin standards need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming. For samples containing lower levels of transferrin, it is possible to extend the utility of the lower detection limit of this assay by making a 2-fold dilution of standard # 1.

Standard	ng/mL	Volume added to 1X Diluent	Volume of 1X Diluent
6	100	10 µL Mouse Transferrin Calibrator	735 µL
5	50	300 µL standard 6	300 µL
4	25	300 µL standard 5	300 µL
3	12.50	300 µL standard 4	300 µL
2	6.25	300 µL standard 3	300 µL
1	3.13	300 µL standard 2	300 µL
0	0		600 µL

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Pipette 100 µL of

   Standard 0 (0.0 ng/mL) in duplicate

   Standard 1 (3.13 ng/mL) in duplicate

   Standard 2 (6.25 ng/mL) in duplicate

   Standard 3 (12.5 ng/mL) in duplicate

   Standard 4 (25 ng/mL) in duplicate

   Standard 5 (50 ng/mL) in duplicate

   Standard 6 (100 ng/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacture's specifications.

Note: The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

Calculation

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from standard curve. Correct for sample dilution factor to arrive at the Transferrin concentration in original sample.

Performance Characteristics

Indications of instability

If the test is performing correctly, the results observed with the standard solutions should be within 20% of the expected values.

Precautions

1. For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.
2. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
3. Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.
4. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
5. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
6. Do not mix or substitute reagents with those from other lots or sources.