Tf (Rat) ELISA Kit

Catalog Number KA1847

96 assays

Version: 03

Intended for research use only

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Introduction

Background

Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow (1).

Principle of the Assay

The Tf (Rat) ELISA Kit is designed for detection of transferrin in rat plasma and serum samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures rat transferrin in approximately 3 hours. A polyclonal antibody specific for rat transferrin has been pre-coated onto a 96-well microplate with removable strips. Rat transferrin in standards and samples is competed by a biotinylated rat transferrin protein sandwiched by the immobilized antibody and streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Rat Transferrin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat transferrin.	96 (8 x 12) wells
Sealing Tapes: Pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Rat Transferrin Standard: Rat Transferrin in a buffered protein base, lyophilized.	16 µg
Biotinylated Rat Transferrin Protein (3x): lyophilized.	1 vial
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water

Precautions for Use

- ✓ This product is for research use only and is not for intended for use in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standards, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial before opening and using contents.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution.

Assay Protocol

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.
- Rat Transferrin Standard: Reconstitute the Rat Transferrin Standard (16 µg) with 1.6 mL of MIX Diluent to generate a 10 µg/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (10 µg/mL) 2-fold with equal volume of MIX Diluent to produce 5, 2.5, 1.25, 0.625 and 0.313 µg/mL solutions. MIX Diluent serves as the zero standard (0 µg/mL). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[Rat Transferrin] (µg/mL)
P1	1 part Standard (10 µg/mL)	10.000
P2	1 part P1 + 1 part MIX Diluent	5.000
P3	1 part P2 + 1 part MIX Diluent	2.500
P4	1 part P3 + 1 part MIX Diluent	1.250
P5	1 part P4 + 1 part MIX Diluent	0.625
P6	1 part P5 + 1 part MIX Diluent	0.313
P7	MIX Diluent	0.000

- Biotinylated Rat Transferrin protein (3x): Reconstitute the Biotinylated Rat Transferrin protein with 4 mL of MIX Diluent to produce a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. From the stock solution, dilute 3-fold with MIX Diluent to produce a 1x working solution. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.
- SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Sample Preparation

- Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 3000-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The

undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).

- Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 3000-fold sample dilution is suggested into MIX Diluent; however user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

✓ Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A. 4 µL sample: 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A : 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A. 4 µL sample: 396 µL buffer (100x) B. 24 µL of A : 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A : 396 µL buffer (100x) C. 24 µL of B : 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 25 µL of Rat Transferrin standard or sample to each well, and immediately add 25 µL of Biotinylated Rat Transferrin protein to each well (on top of the Standard or sample). Gently tap plate to ensure through mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent material to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit it 4-5 times on absorbent material to completely remove the liquid.

5. Add 50 μL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash the microplate as described above.
7. Add 50 μL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 15 minutes or until the optimal blue color density develops.
8. Add 50 μL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
9. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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Data Analysis

Calculation of Results

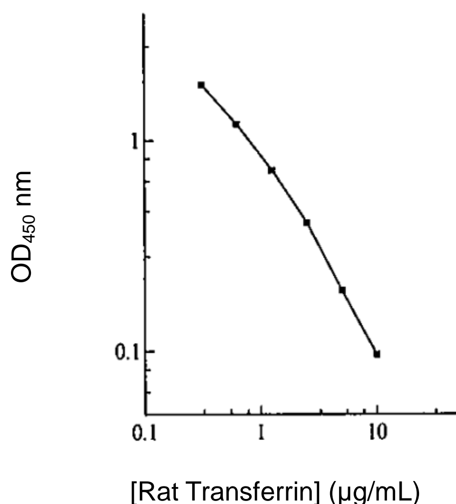
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.
- ✓ Typical Data

  The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	μg/mL	OD	Average OD
P1	10.0	0.099 0.095	0.097
P2	5.0	0.199 0.191	0.195
P3	2.5	0.418 0.398	0.408
P4	1.25	0.731 0.715	0.723
P5	0.625	1.213 1.201	1.207
P6	0.313	1.857 1.837	1.847
P7	0.000	2.338 2.272	2.305
Sample: Pooled Sodium Citrate Rat Plasma (3000x)		0.887 0.859	0.873

- ✓ Standard Curve

  The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



- ✓ Reference Value

  Rat plasma and serum samples were tested (n=20). On average, rat transferrin level was 2820 μg/mL.

Sample	n	Average Value (μg/mL)
Pooled Rat Plasma	10	2423
Pooled Rat Serum	10	3217

Performance Characteristics

- ✓ The minimum detectable dose of rat transferrin as calculated by 2SD from the mean of a zero standard was established to be 0.25 μg/mL.
- ✓ Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- ✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	5.6%	5.4%	5.5%	10.1%	9.8%	10.4%
Average CV (%)	5.5%			10.1%		

- ✓ Linearity

  Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1500x	107%	105%
3000x	97%	98%
6000x	91%	95%

✓ Recovery

Standard Added Value	1.25 – 5 µg/mL
Recovery %	89-111 %
Average Recovery %	95 %

Resources

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Step performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

<table>
<tr><td rowspan="5">Deficient Standard Curve Fit</td><td>Non-optimal sample dilution</td><td>Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay.
Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay.
User should determine the optimal dilution factor for samples.</td></tr>
<tr><td>Contamination of reagents</td><td>A new tip must be used for each addition of different samples or reagents during the assay procedure.</td></tr>
<tr><td>Contents of wells evaporate</td><td>Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.</td></tr>
<tr><td>Improper pipetting</td><td>Pipette properly in a controlled and careful manner.
Check pipette calibration.
Check pipette for proper performance.</td></tr>
<tr><td>Insufficient mixing of reagent dilutions</td><td>Thoroughly agitate the lyophilized components after reconstitution.
Thoroughly mix dilutions.</td></tr>
</table>

References

1. Averbukh Z et. al. (2004) J Nephrol. 17(1): 101-6

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												