ELISA Kit Components	Amount	Part No.
Anti-Human transferrin Microwell Strip Plate	8-well strips (12)	1211
Human Transferrin Control	0.65 ml	1212
Human Transferrin Standard 10 ng/ml	0.65 ml	1213A
Human Transferrin Standard 20 ng/ml	0.65 ml	1213B
Human Transferrin Standard 50 ng/ml	0.65 ml	1213C
Human Transferrin Standard 100 ng/ml	0.65 ml	1213D
Human Transferrin Standard 200 ng/ml	0.65 ml	1213E
Anti-Human Transferrin HRP Conjugate (100X)	0.15 ml	1214
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-1710

Other ELISA kits available from ADI

Human: BD-1, BD-2, BD-3 **and**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgG1, IgG4, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, Leptin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP
Chicken: IgG, IgM, IgY, Ovalbumin
Rabbit: CRP, IgG
Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

Instruction Manual No. M-1210

Human Transferrin

ELISA Kit Cat. No. 1210

For Quantitative Determination of Transferrin in Human Serum

ALPHA DIAGNOSTIC INTERNATIONAL

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INTENDED USE

The Alpha Diagnostics Int'l Human Transferrin ELISA Kit is an immunoassay suitable for quantifying circulating serum transferrin in humans, for use in the research of human iron transport conditions and diseases. The assay can be adapted to measure transferrin in other biological fluids and solutions such as plasma, urine, and culture medium with proper control for assay compatibility

RESEARCH USE OF THE TEST

Elemental iron is required for a variety of normal cellular functions and vital for proper growth and development. However, natural iron is quite insoluble and excess iron is harmful, since it can catalyze the formation of potentially damaging reactive oxygen compounds. Humans have very limited capacity to excrete iron and cells have, therefore, developed mechanisms to improve solubility of iron and to control intracellular iron levels at the point of absorption in the intestine and other tissue. The major pool of body iron (~85%; 40-50 mg/kg) is found in circulating hemoglobin and muscle myoglobin. Several proteins including transferrin, transferrin receptors (TfRs), ferritin and iron regulatory proteins (IRPs) play a key role in iron metabolism.

Transferrin is a serum glycoprotein of ~80 kDa, synthesized in the liver, and is the primary protein of inter-organ transport of nonheme iron. It can bind two iron atoms and is normally about 30% iron-saturated to prevent accumulation of toxic iron. Transferrin (Tf) binds to TfRs and taken up by endocytosis. Iron is released within acidic endosomes into the cytoplasm, apparently through the action of DMT1. The apoTf-TfR complex is returned to the cell surface, where apo-Tf dissociates from TfR at the extra-cellular pH.

PRINCIPLE OF THE TEST

The Human Transferrin ELISA kit is based on the binding of human transferrin in samples to two antibodies, one immobilized on the microwells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of transferrin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The concentration of transferrin in samples and control is calculated from a curve of standards containing known concentrations of transferrin.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Specificity
The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with Transferrin, and have essentially no reactivity with immunoglobulins or any other human serum proteins.

Sera from rhesus and cynomolgous monkeys, baboons and chimpanzees showed extensive reactivity in the ELISA, and serum dilution curves were linear. Therefore, ADI's Human Transferrin ELISA kit may be adapted to measure transferrin in rhesus and cynomolgous monkeys, baboons and chimpanzees.

Serum Transferrin from the following species showed no significant reactivity at 1:100 serum dilution: mouse, rat, hamster, guinea pig, bovine, pig, sheep, rabbit or chicken.

Normal Range
Assay values of transferrin in sera from 30 adult humans ranged from 3.0 to 5.2 mg/ml (average 4.30 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision
Samples containing low, medium and high concentrations of transferrin, representing 3 different sera, were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

Sample	Transferrin ng/ml	Intra-assay %CV	Inter-assay %CV
Human A	115	**2.2**	**8.3**
Human B	292	**4.5**	**6.6**
Human C	518	**8.0**	**6.2**

Linearity of Dilution
Five individual human, chimp and monkey sera were diluted to 2 levels for testing, and concordance of the assay values were compared. Human and chimp had mean recovery of 98%, demonstrating linear dilution and equivalent quantitation across the standard range. The three monkey sera also had good recoveries, 79 to 92%.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Human	1:15k 1:60k	395 95	5.92 5.69	**98 %**
Chimp	1:10k 1:40k	453 109	4.53 4.37	**98 %**
Rhesus	1:5k 1:20k	260 100	1.30 2.01	**79 %**
Cynomolgous	1:5k 1:20k	445 130	2.22 2.60	**92 %**
Baboon	1:5k 1:20k	389 140	1.95 2.80	**82%**

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, transferrin concentrations may be determined as follows:

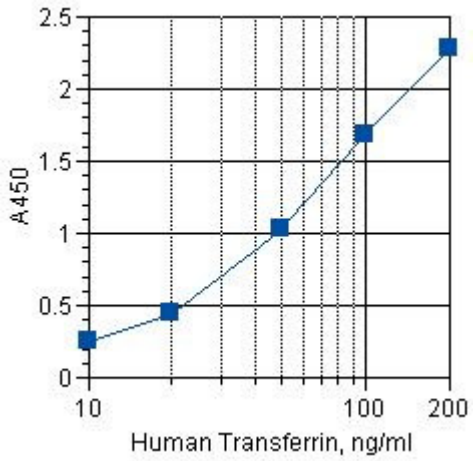
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of transferrin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The transferrin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	**Trf** ng/ml
A1, A2	**Negative Diluent Control**	0.03	0
B1, B2	10 ng/ml **Standard**	0.25	10
C1, C2	20 ng/ml **Standard**	0.45	20
D1, D2	50 ng/ml **Standard**	1.03	50
E1, E2	100 ng/ml **Standard**	1.68	100
F1, F2	200 ng/ml **Standard**	2.28	200
G1, G2	**Positive Serum Control** [Value: 49 – 91 ng/ml]	1.30	66
H1, H2	**Sample** [Diluted 1:20k]	0.59	25
	Calculated: 20k dilution x 25 ng/ml = **0.50** mg/ml in serum		

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Human Transferrin - HRP Conjugate Concentrate (100x) Part No. 1214, 0.15ml	Peroxidase conjugated anti-human transferrin in buffer with protein, detergents and antimicrobials as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Human Transferrin Microwell Strip Plate	1211	8-well strips (12)	Coated with purified anti-human transferrin antibodies.
Human Transferrin Standards			
10 ng/ml 20 ng/ml 50 ng/ml 100 ng/ml 200 ng/ml	1213A 1213B 1213C 1213D 1213E	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing human serum with calibrated transferrin concentrations; diluted in buffer with protein, detergents and antimicrobials as stabilizers.
Positive Control [Transferrin] range on label	1212	0.65 ml	Human serum with stated transferrin concentration range; diluted in buffer with protein, detergents and antimicrobials as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human Transferrin-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately , store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.
The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls, and dispose of these samples and containers as biohazard waste.

Standards, Controls, Sample Diluent, and HRP Antibody contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.
MSDS for TMB, sulfuric acid and BND, if not already on file, can be requested or obtained from the ADI website.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with a Trf concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of 1:10k–1:40k are appropriate for most normal rat sera. For accuracy, two dilution steps are recommended, as follows:
1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:100] + 990ul diluent = **[1:5k].**

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-human Transferrin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.
  Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, assuring the top standard does not surpass 2 OD.

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.