ab108902

Transferrin Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Transferrin concentrations in cell culture supernatants, milk, saliva and urine

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

Version: 2 Last Updated: 05 April 2013
Table of Contents

1. Introduction 3
2. Assay Summary 4
3. Kit Contents 5
4. Storage and Handling 6
5. Additional Materials Required 6
6. Limitations 7
7. Technical Hints 8
8. Preparation of Reagents 10
9. Assay Method 13
10. Data Analysis 15
11. Specificity 18
12. Troubleshooting 19
1. Introduction

Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. Low Transferrin level in plasma could associate with anemia, and chronic liver disease. On the other hand, high plasma Transferrin level could indicate iron deficiency anemia.

ab108902 Transferrin Human ELISA kit is designed for detection of Human Transferrin in urine, milk, saliva, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures Transferrin in less than 4 hours. A polyclonal antibody specific for Transferrin has been pre-coated onto a microplate. Transferrin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Transferrin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 50 μl standard or sample to each well.
Incubate 2 hours at room temperature.

Wash the microplate 5 x with wash buffer.

Add 50 μl prepared biotin antibody to each well.
Incubate 1 hour at room temperature.

Wash the microplate 5 x with wash buffer.

Add 50 μl of Streptavidin-Peroxidase Conjugate.
Incubate 30 minutes at room temperature.

Wash the microplate 5 x with wash buffer.

Add 50 μl of Chromogen Substrate to each well.
Incubate 10 minutes or till the optimal blue colour density develops.

Add 50 μl Stop Solution to each well. Read at 450 nm immediately.
3. Kit Contents

- Human Transferrin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against Human Transferrin.
- Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Biotinylated Transferrin Antibody (50x): A 100-fold concentrated biotinylated polyclonal antibody against Transferrin (140 µl).
- Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml).
- Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µl)
- Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
4. Storage and Handling

Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.

Store SP Conjugate and Biotinylated Antibody at -20°C

Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C

Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Diluent (1x) may be stored for up to 1 month at 2-8°C.

Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 450nm.
- Precision pipettes to deliver 1 μl to 1 ml volumes.
- Distilled or deionized reagent grade water.
6. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures

- Do not use kit or components if it has exceeded the expiration date on the kit labels

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
7. Technical Hints

- It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.

- The coefficient of determination of the standard curve should be ≥ 0.95 and the highest O.D. should be more than 1.0.

- Cover or cap all kit components and store at 2-8°C when not in use.

- Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.

- Samples should be collected in pyrogen/endotoxin-free tubes.

- When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.

- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
• Do not mix or interchange different reagent lots from various kit lots.

• Read absorbance immediately after adding the stop solution.

• Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

• Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.
8. Preparation of Reagents

Sample Collection:

1. **Cell culture supernatants**: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

2. **Urine**: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Urine dilution is suggested at 1:4 in Diluent; however, the user should determine the optimal dilution factor. Store undiluted samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

3. **Saliva**: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Saliva dilution is suggested at 1:200 into Diluent; however, the user should determine the optimal dilution factor. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

4. **Milk**: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Milk dilution is suggested at 1:400 in Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Reagent Preparation:

1. Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

2. **Diluent (10x):** Dilute the Diluent 1:10 with reagent grade water. Store for up to 1 month at 2 to 8°C.

3. **Standard Curve:** Reconstitute the Transferrin Standard with the appropriate amount of Diluent to generate a standard solution of 400 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (400 ng/ml) 1:4 with Diluent to produce 100 ng/ml. Create the standard curve by serially diluting 1:2 with Diluent to produce 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/ml solutions. Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.
### Standard Dilution [H. Transferrin] (ng/ml)

<table>
<thead>
<tr>
<th>Standard Point</th>
<th>Dilution</th>
<th>[H. Transferrin] (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Standard (400 ng/ml) + 3 parts Diluent</td>
<td>100.000</td>
</tr>
<tr>
<td>P2</td>
<td>1 part P1 + 1 part Diluent</td>
<td>50.000</td>
</tr>
<tr>
<td>P3</td>
<td>1 part P2 + 1 part Diluent</td>
<td>25.000</td>
</tr>
<tr>
<td>P4</td>
<td>1 part P3 + 1 part Diluent</td>
<td>12.500</td>
</tr>
<tr>
<td>P5</td>
<td>1 part P4 + 1 part Diluent</td>
<td>6.250</td>
</tr>
<tr>
<td>P6</td>
<td>1 part P5 + 1 part Diluent</td>
<td>3.125</td>
</tr>
<tr>
<td>P7</td>
<td>1 part P6 + 1 part Diluent</td>
<td>1.563</td>
</tr>
<tr>
<td>P8</td>
<td>Diluent</td>
<td>0.000</td>
</tr>
</tbody>
</table>

4. **Biotinylated Transferrin Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with Diluent. Any remaining solution should be frozen at -20°C.

5. **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

6. **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent. Any remaining solution should be frozen at -20°C.
9. Assay Method

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°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 50 µl of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

5. Add 50 µl of Biotinylated Transferrin Antibody to each well and incubate for 1 hour.

6. Wash microplate as described above.

7. Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

8. Wash microplate as described above.
9. Add 50 μl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

10. Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.

11. 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 high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
10. Data Analysis

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 on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

A. Typical Data

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

The minimum detectable dose of Transferrin is typically 1.5 ng/ml.

C. **Recovery**

Standard Added Value: 5 – 50 ng/ml

Recovery %: 85 – 112

Average Recovery %: 96

D. **Reproducibility**

Intra-Assay: CV = 4.7%

Inter-Assay: CV = 7.2%
### E. Linearity

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Average Percentage of Expect Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>1:2</td>
<td>94</td>
</tr>
<tr>
<td>1:4</td>
<td>98</td>
</tr>
<tr>
<td>1:8</td>
<td>101</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Average Percentage of Expect Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saliva</td>
</tr>
<tr>
<td>1:100</td>
<td>83</td>
</tr>
<tr>
<td>1:200</td>
<td>95</td>
</tr>
<tr>
<td>1:400</td>
<td>102</td>
</tr>
<tr>
<td>1:800</td>
<td></td>
</tr>
</tbody>
</table>
11. Specificity

10% FBS in culture media will not affect the assay.

<table>
<thead>
<tr>
<th>Species</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>None</td>
</tr>
<tr>
<td>Mouse</td>
<td>None</td>
</tr>
<tr>
<td>Rat</td>
<td>None</td>
</tr>
<tr>
<td>Swine</td>
<td>None</td>
</tr>
<tr>
<td>Monkey</td>
<td>&lt; 5 (suggest 1:2 dilution for plasma)</td>
</tr>
<tr>
<td>Beagle</td>
<td>None</td>
</tr>
</tbody>
</table>
## 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaw cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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