



For better life

Human Transferrin Antigen ELISA Kit

Cat. No. E20142006 (One 96-well Plate, 12 x 8-well strips)

America Diagnostics' AmeriDx® Human Transferrin Antigen ELISA Kit is designed for the quantification of recombinant and natural human Transferrin in solutions including cell culture supernatant, serum, and other biological fluids. The assay is sensitive, accurate, and reliable.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Human Transferrin Capture Antibody coated ELISA plate, 12 x 8-well strips (1)
2. Biotinylated conjugated Human Transferrin Detection Antibody, 15 mL (1)
3. Human Transferrin Standards:
 - CAL A Transferrin 0 ng/mL, 8 mL (1)
 - CAL B Transferrin 12.3 ng/mL, 0.75 mL (1)
 - CAL C Transferrin 37.0 ng/mL, 0.75 mL (1)
 - CAL D Transferrin 111.1 ng/mL, 0.75 mL (1)
 - CAL E Transferrin 333.3 ng/mL, 0.75 mL (1)
4. 20X Avidin-HRP, 0.75 mL (1)
5. 10X Wash Buffer Concentrate, 25 mL (1)
6. ELISA Substrate Solution, 12 mL (1)
7. Stop Solution, 12 mL (1)

Introduction

Transferrin is a type of $\beta 1$ globulin with a molecular weight of 77 KD, responsible for transferring extracellular iron into cells through cell membrane receptor-mediated endocytosis. Transferrin is more stable than hemoglobin. When gastrointestinal bleeding occurs, serum transferrin enters the gastrointestinal tract and is excreted in the feces. For this reason, Transferrin is a potential protein marker for a number of epithelial cancers including colorectal cancer.

Principle of the Test

America Diagnostics' AmeriDx® Human Transferrin Antigen ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay. A human Transferrin specific mouse monoclonal antibody is first coated on a 96-well plate. Standards and samples are then added to the wells. The Transferrin binds to the immobilized capture antibody. Next, a biotinylated mouse monoclonal anti-human Transferrin detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase (Avidin-HRP) is subsequently added, followed by ELISA Substrate Solution, producing a blue color.

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The blue color intensity is proportional to the concentration of Transferrin present in the sample. Finally, the addition of the Stop solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm and 650 nm with a microplate reader.

Materials to be Provided by the End-User

- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Adjustable pipettes to measure volumes ranging from 2 μ L to 1 mL
- Software for data analysis (or Microsoft Excel, or Openoffice calc etc.)
- Tubes to prepare standard dilutions
- Reservoir for diluted wash buffer
- Timer
- Absorbent paper

Storage Information

- Store the kit between 2°C and 8°C.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to appropriate storage conditions.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at $< -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube, wait at least 30 minutes to allow clotting. Centrifuge for 10 minutes at 1,000 X g, then remove serum layer. Assay immediately or store serum samples at $< -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood sample in a citrate, heparin, or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X g within 30 minutes of collection. Assay immediately or store plasma samples at $< -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Stool Antigen Extraction:

1. Unscrew the fecal sample bottle (Cat# ADX9020), use the applicator stick attached to the cap to transfer small piece of stool (5-6 mm in diameter; approximately 100 mg – 200 mg/0.1-0.2 g) into the sample bottle containing specimen preparation buffer.
2. Replace the stick in the bottle and tighten securely. Thoroughly mix the stool sample with the buffer by shaking the bottle for a few seconds.
3. Hold the fecal sample bottle upright with the tip point in the direction away from the test performer then snap off the tip.
4. Hold the bottle in a vertical position over the sample well of the test card and deliver 4-6 drops (400 -600 μ L) of diluted stool sample to an Eppendorf tube. Centrifuge for 5 minutes at 1000 x g and transfer the supernatant to a new tube to be used for the ELISA assay.

Reagents Prepared Before Assay

1. Reagents that contain preservatives may be harmful if ingested, inhaled, or absorbed through the skin. Refer to the MSDS online for details (www.ameridix.com/msds).
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.
3. Dilute the 10X Wash Buffer Concentrate 1:10 using Deionized (DI) water.
4. Dilute Avidin-HRP 1:20 using 1X Wash Buffer. For one plate, dilute 550 μ L of Avidin-HRP with 11 mL of 1X Wash Buffer.
5. **Samples:** For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilution is required, samples should be diluted with CAL 0 Standard before adding to the wells. It is recommended that serum, plasma, and fecal samples be diluted 20-fold with CAL 0 Standard due to high endogenous levels.

Assay Procedure

Do not use sodium azide in any solutions, as it inhibits the activity of the horseradish-peroxidase enzyme.

1. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in triplicate. A standard curve is required for each assay.
2. Wash plate 4 times with at least 250 μ L of Wash Buffer per well. Blot residual buffer by firmly tapping the plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
3. If necessary, use the CAL 0 Standard to dilute the samples.
4. Add 25 μ L/well of standards and samples to the appropriate wells. Then add 75 μ L of Wash Buffer to all the wells so that each well has a total volume of 100 μ L.
5. Seal the plate and incubate at RT for 2 hours with shaking (e.g. 500 rpm) **All subsequent incubation with shaking should be performed similarly.**
6. Wash plate 4 times with Wash Buffer.
7. Add 100 μ L of biotinylated Detection Antibody solution to each well.
8. Seal the plate and incubate at RT for 1 hour with shaking.
9. Wash plate 4 times with Wash Buffer.
10. Add 100 μ L of diluted Avidin-HRP solution to each well.
11. Seal the plate and incubate at RT for 30 minutes with shaking.
12. Wash plate 5 times with Wash Buffer. For these final washes, soak the wells in Wash Buffer for 30 seconds to 1 minute with shaking. This will minimize background.
13. Add 100 μ L of ELISA Substrate Solution.
14. Incubate **in the dark** for 15 minutes*. Positive wells should turn blue in color. It is not necessary to seal or shake the plate during this step.
15. Stop reaction by adding 100 μ L of Stop Solution to each well. Positive wells should turn from blue to yellow.

16. Read absorbance at 450 nm within 15 minutes. If the reader can read at 650 nm, the absorbance at 650 nm can be subtracted from the absorbance at 450 nm.

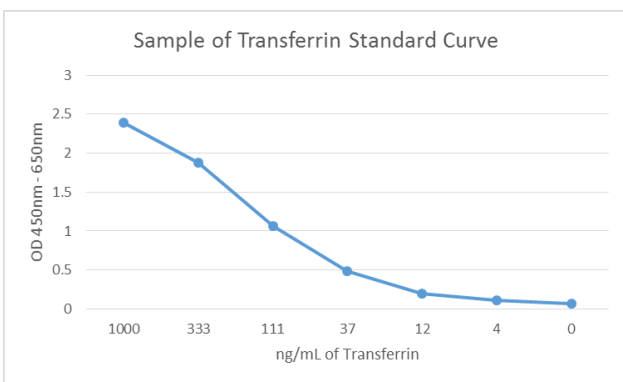
***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Calculation of Results

1. Plot the standard curve on Microsoft Excel sheet, with analyte concentration on the x-axis and absorbance on the y-axis.
2. Click on the data points on the graph with the mouse cursor, right click to display the menu. Then add "Trendline" on the chart and check "Display the equation on chart". Use this equation to calculate the unknown analyte's concentration in the samples.
3. If the samples were diluted, multiply by the appropriate dilution factor.
4. If the absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at an appropriate higher or lower dilution.
5. The data can also be applied in other statistic/graphic software to calculate the unknown analyte's concentration.

Typical Data

Standard Curve: This standard curve was generated at America Diagnostics for demonstration purposes only. A standard curve must be run with each assay.



Assay Procedure Summary

1. Wash 4 times
Add 25 μ L standards and samples + 75 μ L Wash Buffer.
Incubate 2 hrs, RT
2. Wash 4 times
Add 100 μ L biotinylated Detection Antibody. Incubate 1 hr, RT
3. Wash 4 times
Add 100 μ L Avidin-HRP. Incubate 30 min. RT
4. Wash 5 times
Add 100 μ L ELISA Substrate
Incubate 15 min. RT, in the dark
5. Add 100 μ L Stop Solution
6. Read absorbance at 450 nm and 650 nm

Performance Characteristics

Sensitivity: The minimum detectable concentration of Transferrin Antigen for this kit is 5 ng/mL

Specificity: N / A

Troubleshooting

High Background:

1. Background wells were contaminated.
2. Matrix used had endogenous analyte.
3. Plate was insufficiently washed.
4. ELISA Substrate Solution was contaminated.

No signal:

5. Incorrect or no antibodies were added.
6. Avidin-HRP was not added.
7. ELISA Substrate solution was not added.
8. Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

9. Standard was incompletely reconstituted.
10. Standard was incomplete or was stored improperly.
11. Wash buffer contains sodium azide.
12. Reagents were added to wells with incorrect concentrations.
13. Plate was incubated with improper temperature, timing, or agitation.

Signal too high, standard curve saturated:

14. Standard was reconstituted with less volume that required.
15. One or more reagent incubation step was too long.
16. Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

17. Samples contain no or below detectable levels of analyte.
18. Samples contain analyte concentrations greater than the highest standard point.

High variations in samples and/or standards:

19. Pipetting errors may have occurred.
20. Plate washing was inadequate or nonuniform.
21. Samples were not homogenous.
22. Samples or standard wells were contaminated.

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