

ab284010 – Human Lactoferrin Autoantibody ELISA Kit

For the quantitative measurement of Human Lactoferrin Autoantibody in human plasma and serum samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab284010

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the SP Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
10X Diluent N Concentrate	30 ml	4°C
20X Wash Buffer Concentrate	2 x 30 ml	4°C
Chromogen Substrate	7 mL	4°C
Human Lactoferrin Autoantibody Microplate	96 wells	-21°C
Lactoferrin Autoantibody Standard	1 vial	4°C
Sealing Tapes	3 units	4°C
Stop Solution	11 mL	4°C
40X Biotinylated Human Lactoferrin Autoantibody Antibody	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Freshly dilute all reagents and bring all reagents to room temperature before use.

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

Prepare only as much reagent as is needed on the day of the experiment.

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent N Concentrate: Dilute the Diluent N Concentrate 1:10 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.

40X Biotinylated Mouse GSTA1 Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 1:40 with Diluent N to produce a 1x solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

100X Streptavidin-Peroxidase Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent N to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.

Prepare serially diluted standards immediately prior to use.

Any remaining standard should be stored at -20°C after reconstitution and used within 30 days. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

Reconstitution of the Lactoferrin Autoantibody Standard vial to prepare a 100 AU/ml Stock Standard:

- First consult the Lactoferrin Autoantibody Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X Diluent N Concentrate to add when resuspending the Lactoferrin Autoantibody Standard vial to produce a 100 AU/ml Lactoferrin Autoantibody Stock Standard by using the following equation:
 - o C_s = Starting mass of Lactoferrin Autoantibody Standard (see vial label) (AU)
 - o C_f = The 100 AU/ml Lactoferrin Autoantibody Stock Standard final required concentration
 - o V_d = Required volume of 1X Diluent N Concentrate for reconstitution (µL)
 - o Calculate total required volume 1X Diluent N Concentrate for resuspension:
$$(C_s / C_f) \times 1,000 = V_d$$

Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_s = 200 AU of Human Lactoferrin Autoantibody Standard in vial

C_f = 100 AU/mL Human Lactoferrin Autoantibody Standard #1 final concentration

V_d = Required volume of 1X Diluent N for reconstitution (200 AU / 100 AU/mL) x 1,000 = 2000 µL

- Reconstitute the Human Lactoferrin Autoantibody Standard vial by adding the appropriate calculated amount V_d of 1X Diluent N to the vial to generate the 100 AU/mL Human Lactoferrin Autoantibody Standard #1. Mix gently and thoroughly.
- Allow the reconstituted 100 AU/mL Albumin Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label five tubes #2 – 8.
- Add 120 µL of 1X Diluent N to tube #2 – 8.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.
- To prepare Standard #3, add 120 µL of the Standard #2 into tube #3 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent N serves as the zero standard (0 AU/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent N Concentrate (µL)	Lactoferrin (AU/mL)
1	240	-	100
2	120 µL Standard #1	120	50
3	120 µL Standard #2	120	25
4	120 µL Standard #3	120	12.5
5	120 µL Standard #4	120	6.25
6	120 µL Standard #5	120	3.125
7	120 µL Standard #6	120	1.563
8	-	120	0.0

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 40-fold sample dilution is suggested into Diluent N; 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 an 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 40-fold sample dilution is suggested into Diluent N; 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.

Δ Note: Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed, and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls, and samples in duplicate

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 desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator at -20°C.
3. Add 50 µl of Lactoferrin Autoantibody Standard or sample 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 2 hours. Start the timer after the last addition.

4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.⁶
5. Add 50 µl of Biotinylated Human Autoantibody Antibody 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 1 hour.
6. Wash the microplate as described above.
7. 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.
8. Wash the microplate as described above.
9. 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 20 minutes or until the optimal blue color density develops.
10. 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.
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.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. 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 log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the standard curve.
4. Although normal samples have been diluted 40-fold, do not multiply the value by the dilution factor. Samples with elevated levels of autoantibodies can be diluted further; for example: 80x. Account for this further dilution factor when calculating the value of the sample.

TYPICAL DATA

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

Standard Point	AU/ml	Average OD
P1	100	1.945
P2	50	1.570
P3	25	1.120
P4	12.5	0.895
P5	6.25	0.618
P6	3.125	0.377
P7	1.563	0.224
P8	0.0	0.044
40X Normal Level Sample: Serum with normal level of anti-Lactoferrin IgG		0.523
80X Elevated Level Sample: Serum with elevated level of anti-Lactoferrin IgG		1.021

PERFORMANCE CHARACTERISTICS

- The minimum detectable dose of anti-Lactoferrin IgG as calculated by 2SD from the mean of a zero standard was established to be 0.31 AU/ml.
- Intra-assay precision was determined by testing three serum samples twenty times in one assay.
- Inter-assay precision was determined by testing three serum samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	5.6	9.3

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

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