ELISA kits available from ADI (see details at the web site)

3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-
3210-SSA	Human anti-SS-A/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Human Anti-thryroid peroxidase ELISA kit, Semi-Quantitative
3300	Human Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-APS	Human Anti-Phospholipid Screen IgG/IgM ELISA kit, 96 tests,
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests,
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests,
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP)IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests,
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative
3310	Human Anti-helicobacter pylori IgM ELISA kit, Semi-Quantitative
3320	Human Anti-helicobacter pylori IgA ELISA kit, Semi-Quantitative
3600-HIG	Human Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3700-MIG	Mouse Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3710-MIM	Mouse Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
3750-RIG	Rat Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3760-RIM	Rat Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
4000	Mouse Anti-Myelin Oligodendrcyte protein (MOG35-55) Ig's ELISA kit,

Instruction Manual No. M-3300-260-LFG

Human Anti-Lactoferrin IgG ELISA

ELISA KIT Cat. No. 3300-260-LFG

For Quantitative Determination of autoantibodies against Lactoferrin in human serum, or plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com
Web Site: www.4adi.com

DRAFT MANUAL: USE THE MANUAL SUPPLIED WITH THE KIT FOR LOT SPECIFIC CHANGES.

Human Anti-Lactoferrin IgG ELISA KIT Cat. No. 3300-260-LFG

For Quantitative Determination of autoantibodies against Lactoferrin

Kit Contents: (reagents for 96 tests)

Components	
Highly purified Lactoferrin Coated microwell (8 x 12 strips), Ready-to-use, #3 3 0 0 - 2 6 1 P	1 Plate
Anti-Lactoferrin IgG **Std A**, 0 U/ml; 1.5 ml, #3 3 0 0 2 6 2 A	1 vial
Anti-Lactoferrin IgG **Std B**, 6.3 U/ml; 1.5 ml, #3 3 0 0 2 6 2 B	1 vial
Anti-Lactoferrin IgG **Std C**, 12.5 U/ml; 1.5 ml, #3 3 0 0 2 6 2 C	1 vial
Anti-Lactoferrin IgG **Std D**, 25 U/ml; 1.5 ml, #3 3 0 0 2 6 2 D	1 vial
Anti-Lactoferrin IgG **Std E**, 50 U/ml; 1.5 ml, #3 3 0 0 2 6 2 E	1 vial
Anti-Lactoferrin IgG **Std F**, 100 U/ml; 1.5 ml, #3 3 0 0 2 6 2 F	1 vial
Stds are calibrated against the WHO refs WO/80.	
Anti-Lactoferrin IgG **Positive control,** 1.5 ml, #3 3 0 0 2 6 0 P	1 vial
Anti-Lactoferrin IgG **Negative control,** 1.5 ml, #3 3 3 0 0 2 6 0 N	1 vial
Sample Buffer (5X), 20 ml (yellow), Dilute stock to a final volume of 100 ml distilled water #3 3 0 0 2 6 3	1 bottle
Anti-hIgG HRP-**Conjugate**, 15 ml, #3 3 0 0 2 6 4	1 bottle
Wash Buffer (50X); 20 ml, **dilute 1:50 with distilled water, #** 3 3 0 0 2 6 0 - W B	1 bottle
HRP Substrate **Solution**, 15 ml, # 3 3 0 0 2 6 0 T M	1 bottle
Stop Solution, 15 ml, # 3 3 0 0 2 6 0 - S T	1 bottle
Complete Instruction Manual; 3300-260-LFG	1

Intended Use

ADI's Human Anti-Lactoferrin IgG ELISA KIT Test Kit has been designed for the detection autoantibodies against Lactoferrin in human serum or plasma. This kit is for in vitro research use only (RUO).

INTRODUCTION: need new info

The acronym ANCA (Antineutrophil Cytoplasmic Autoantibodies) is defined by an accumulation of autoantibodies with specificity against different granulocytic, monozytic and probably endothelial cytoplasmic antigens. PR3 and MPO are well defined as reliable serological markers for a definite group of primary systemic vasculitides (PSV), which were also named ANCA associated vasculitides (AAV). The occurrence of AAV is clearly higher than supposed. The incidence is 1.5 per 1000 and in the group of older persons nearly 5 per 1000. The clinical appearance of the AAV is characterized trough manifestations in lung, kidney and respiratory tract. In the last years, newer investigations discovered and characterized a couple of new pANCA antigens: Elastase, Cathepsin G, Lysozyme and Lactoferrin. Up to now, ANCA screening has been done with immunofluorescence techniques, but often there have been difficulties in the evaluation and in clinical findings. Therefore, the results have to be scrutinized with counter examinations on other cells or in other test systems like ELISA. Moreover it was not possible to differentiate the single cANCA and pANCA antigens.

Interpretation of results

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Std A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not met, the results are invalid and the test should be repeated

Parallelism:

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Lactoferrin kit. The assay showed linearity over the full measuring range.

Sensitivity:

The lower detection limit for the Anti-Lactoferrin test was determined at **0.5 U/ml.**

Specificity:

The microplate is coated with Lactoferrin. The antigen preparation is highly purified by affinity chromatography. The Anti-Lactoferrin test is specific only for autoantibodies directed against anti- Lactoferrin. No crossreactivities to the other ANCA antigens have been observed.

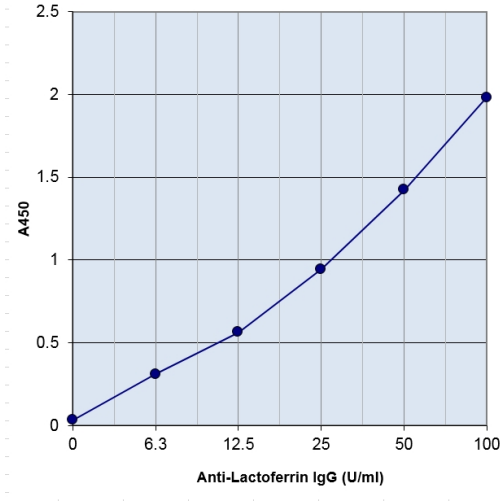
Calibration:

Since no international reference preparations for Anti-Lactoferrin autoantibodies is available, the assay system is calibrated in arbitrary units.

References: Tan L, J Immunoassay Immunochem. 2014;35(4):388-97; Bracci F, Hayakawa T, Intern Med. 2009;48(15):1251-4; Jin CX (2009) JOP. 2009 May 18;10(3):237-41; Caccavo D (2005) Iin Rheumatol. 24(4):381-7,

WORKSHEET OF TYPICAL ASSAY

Wells	Controls /samples	mean $A_{450\ nm}$	Calculated Concn. (IU/ml)*
A1, A2	**Sample Diluent**(Blank)		
B1, B2	**Std A (0 U/ml)**		
C1, C2	**Std B (6.3 U/ml)**		
D1, D2	**Std C (12.5 U/ml)**		
E1, E2	**Std D (25 U/ml)**		
F1, F2	**Std E (50 U/ml)**		
G1, G2	**Std F (100 U/ml)**		



1-ADI-ELISA-Arif

NOTE: A complete set of positive controls and standards must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values, particularly the cut-off control values for its own sub-population.

Calculation of results:

For Anti-Lactoferrin IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot:

First calculate the averaged optical densities for each std well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all stds points. The stds points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

PRINCIPLE OF THE TEST

Highly purified Lactoferrin is bound to microwells . Antibodies to this antigen, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically bind to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable (5-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, wash bottle or ELISA plate Washer and Reader.

PRECAUTIONS

This ELISA kit is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The negative, positive, and calibrator standards has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Wash buffer(50X): Dilute (1:50) with distilled water (20 ml stock to final volume of 1000 ml). Store at 4oC.

Sample Buffer: Dilute 5X stock to a final volume of 100 ml distilled water.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8ºC until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate conditions. **Do not** contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE).*

Dilute wash buffer (1:50) with distilled water (20 ml stock to final volume of 1000 ml) & Sample Buffer: Dilute 5X stock to a final volume of **100 ml distilled water**.

1. **Dilute** serum samples (1:100 with sample buffer). .Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.
2. Pipet **100 ul** of *Standards, and* positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently, cover the plate and incubate for **30 minutes** at 28-30 ºC.
3. Aspirate and wash the wells **3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. **Failure to wash the wells properly will lead to high blank values.** If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 ul of enzyme conjugate** into each well**.** Mix gently. Cover the plate and incubate for **15 minutes** at room temp.
5. Aspirate and **wash the wells 3 times** as in step3 above.
6. Dispense **100 ul TMB substrate per well**. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature.

7 .Stop the reaction by adding **100 ul** of stop solution to all wells. Mix gently. Measure the absorbance at 450 nm using an ELISA reader within 15 min.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Std A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not met, the results are invalid and the test should be repeated

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established for the Anti-Lactoferrin test:

Anti-Lactoferrin IgG [U/ml]

normal: **< 10**
elevated: **≥ 10**

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Lactoferrin antibodies. The above reference ranges should be regarded as guidelines only.

LIMITATIONS

The Anti-Lactoferrin ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.