Human ANA IgG ELISA Kit

Cat.No:DEIA1695

Lot. No. (See product label)

PRODUCT INFOMATION

Storage	1. Store the unopened kit at 2-8 °C. 2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator remains blue. 3. Conjugate: Store at 2-8 °C. DO NOT FREEZE. 4. Calibrator, Positive Control and Negative Control: Store at 2-8 °C. 5. TMB: Store at 2-8 °C. 6. Wash buffer concentrate (10X): Store at 2-25 °C. Diluted wash buffer (1X) is stable at room temperature (20 to 25 °C) for up to 7 days or for 30 days between 2 and 8 °C. 7. Sample Diluent store between 2 and 8 °C. 8. Stop Solution: Store between 2-25 °C.
specificity	Specimens negative for ANA by Hep-2 IFA and positive for IgG antibody to various antigens such as EBV-VCA, EBNA, HSV-1, HSV-2, CMV, Rubella, and/or Toxo, were tested for potential cross reactivity using the ANA Screen ELISA test system. All samples were negative on the ELISA indicating that the potential for cross reactivity with such antibodies is minimal.
Sensitivity	1. Calculation including equivocal specimens; without resolution of discrepant specimens 141/156 = 90.4 2. Calculation excluding equivocal specimens; after resolution of discrepant specimens 141/147 = 95.9%
Pkg#Size	96T
Intended use	The ANA ELISA test system is a qualitative screening assay designed to detect anti-nuclear antibodies (ANA) in human sera. When performed according to the enclosed instructions, this test system is capable of detecting all ANAs commonly tested for, such as those against double stranded DNA (dsDNA), Jo-1, Sm, Sm/RNP, SSA, SSB, and Scl-70. The test is also capable of detecting ANA demonstrating centromere, nucleolar, peripheral, and spindle indirect immunofluorescence antibody (IFA) patterns.
Principle Of The Test	The ANA Screen ELISA test system is designed to detect IgG class antibodies in human sera to a variety of common nuclear antigens. The test procedure involves three incubation steps: 1. Test sera (properly diluted) are incubated in antigen coated microwells. If present in patient sera, specific antibodies will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components. 2. Peroxidase conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate. 3. The microtiter wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

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Reagents And Materials Provided	Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v). 1. Plate. 96 wells configured in twelve 1x8-well strips coated with inactivated antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant. 2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One, 15mL vial with a white cap. Preservative added. 3. Positive control (Human Serum). One, 0.35 mL vial with a red cap. Preservative added. 4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap. Preservative added. 5. Negative control (Human Serum). One. 0.35 mL vial with a green cap. Preservative added. 6. Sample diluent. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphatebuffered-saline, pH7.2±0.2. Green solution, ready to use. Note: Shake Well Before Use. Preservative added. (NOTE: This reagent may be used with any ELISA test system. 7. TMB: One 15 mL amber bottle (amber cap) containing 3,3', 5, 5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO 8. Stop solution. One 15 mL bottle (red cap) containing 1M H2S04 0.7M HCI. Ready to use. 9. Wash Buffer Concentrate (10X). Dilute one part concentrate ± 9 parts deionized or distilled water. One, 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). Contains preservative. Note: 1X solution will have a pH of 7.2 ± 0.2.
Materials Required But Not Supplied	1. ELISA microwell reader capable of reading at a wavelength of 450nm. 2. Pipettes capable of accurately delivering 10 and 200µL. 3. Multichannel pipette capable of accurately delivering (50-200 uL), 4. Reagent reservoirs for multichannel pipettes. 5. Wash bottle or plate washing system. 6. Distilled or deionized water. 7. One liter graduated cylinder. 8. Serological pipettes. 9. Disposable pipette tips. 10. Paper towels 11. Laboratory timer to monitor incubation steps. 12. Disposal basin and disinfectant, (Example: 10% household bleach, 0.5% sodium hypochlorite).

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Assay Steps

1. Remove the individual kit components from storage and allow them to warm to room temperature (20-25 °C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Bank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 and 8 °C.
3. Prepare a 1:21 dilution (e.g.: 10µL of serum ± 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100 µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25 °C) for 60 to 65 minutes.
7. Wash the microwell strips 5X.

A. MANUAL WASH procedure

a) Vigorously shake out the liquid from the wells.
b) Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
c) Repeat steps a. And b. for a total of five washes.
d) Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmLy to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. AUTOMATED WASH procedure

If using an automated wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmLy to remove any residual wash solution from the microwells.
8. Add 100µL of 1X conjugate solution to each well at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25 °C) for 30 to 35 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100 µL of TMB to each well including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25 °C) for 30 to 3 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and
in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution,
tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microplate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after addition of the stop solution.

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Quality Control	1. Each time the assay is run, the low positive standard (LPS) must be run in triplicate. A high positive and negative control must also be included in each assay. 2. Calculate the mean of the three low positive determinations. If any of the three LPS values differs by more than 15% from the mean, discard that value and calculate the mean of the remaining two values. 3. The mean OD value for the LPS and the OD values for the high positive and negative controls should fall within the following ranges: Negative Control ≤ 0.250 Calibrator ≥ 0.300 Positive Control ≥ 0.500 a) The OD of the negative control divided by the mean OD of the LPS should be ≤ 0.9. b) The OD of the positive control divided by the mean of the Calibrator should be ≥ 1.25. c) If the above conditions are not met, the test should be considered invalid and should be repeated 1. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off. 2. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. 3. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

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Precautions	1. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws. 2. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly. 3. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institute of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20). 4. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use. 5. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations. 6. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (sodium azide, 0.1% (w/v) react with laboratory plumbing which may cause explosion on hammering. 7. The Stop Solution is TOXIC. Causes burn. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately. 8. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin. 9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin. 10. Wipe bottom of plate free of residual liquid and/or fingerprints, which can alter optical density (OD) readings. 11. Dilution or adulteration of these reagents may generate erroneous results. 12. Reagents from other sources or manufacturers should not be used. 13. TMB Solution should be colorless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test procedure, Substrate Incubation section to determine the amount of TMB to be used. 14. Never pipette by mouth. Avoid contact or reagents and patient specimens with skin and mucous membranes. 15. Avoid microbial contamination of reagents. Incorrect results may occur. 16. Cross contamination of reagents and/or samples could cause erroneous results. 17. Reusable glassware must be washed out and thoroughly rinsed free of all detergents. 18. Avoid splashing or generation of aerosols. 19. Do not expose reagents to strong light during storage or incubation. 20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation. 21. Wash solution should be collected in a disposal basin. Treat the waste solution with 10 household bleach (0.% sodium hypochlorite). Avoid exposure to reagents to bleach fumes. 22. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution. 23. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink. 24. Do not allow the conjugate to come in contact with containers or instruments, which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity. 25. Do not expose any of the reactive reagents to bleach-containing solutions, or to any strong odors from bleachcontaining solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.
Specimen Collection And Handling	1. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious. 2. Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera. 3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

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Calculation	Correlation Factor A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each new lot of kit components and is printed on the data label located in the kit box. Cutoff OD Value To obtain the cutoff value, multiply the CF by the mean OD of the Calibrator determined above. Index Values or OD ratios Calculate the Index Value or OD ratio for each sample by dividing its OD value by the cutoff OD from Step 2.
Reference Values	The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested. With respect to disease-state and percent reactivity, Table I in the Significance and Background section for this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.
General Description	In recent years it has become clear that autoantibodies to a number of nuclear constituents have proven to be useful in the diagnosis of various connective tissue diseases. Antibodies to dsDNA are highly specific for active systemic lupus erythematosus (SLE), and correlates closely with the onset of lupus nephritis. The Jo-1 autoantibody is one of a family for characteristic autoantibodies seen in myositis patients. They are all specifically found in patients with myositis, and are associated with a high incidence of accompanying interstitial lung disease. Antibodies directed against the Sm marker are highly specific for patients with SLE and are considered a diagnostic criterion for SLE . The presence of high level RNP antibodies alone are considered diagnostic of mixed connective tissue disease (MCTD) and are usually associated with a more benign disease course, while patients with low levels of RNP antibodies, together with other autoantibodies, may be observed in the serum of patients with progressive systemic sclerosis, Sjogren's Syndrome, and rheumatoid arthritis. The presence of RNP antibodies in the serum of SLE patients is usually associated with a lower incidence of renal involvement and a more benign disease course. To the contrary, patients with Sm antibodies experience a higher frequency of renal and central nervous system complications. Autoantibodies directed against SSA and SSB may be observed in patients with SLE, and Sjogren's disease . SSA antibodies are frequently present in the serum of ANA negative SLE patients, such as subacute cutaneous lupus erythematosus, a lupus-like syndrome associated with a homozygous C2 deficiency, and in a subset of patients who lack anti-dsDNA antibodies. Scl-70 antibodies are highly specific for scleroderma. They are also observed in a minority of SLE patients. Scl-70 positive scleroderma patients tend to have a more severe disease course, more internal organ involvement and diffuse rather than limited skin involvement. Scl-70 antibodies are rarely found in other autoimmune diseases, and thus, their detection in a patient with the recent onset of Raynaud's phenomenon is highly significant. Until recently, autoantibodies were tested individually by indirect immunofluorescence, Ouchterlony gel diffusion, hemagglutination, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). Unlike several other systems, the ELISA methodology offers sensitive objective, and rapid evaluation of specimens, and therefore is suitable for screening a large number of samples for total ANA. Although the exact etiology of autoimmune diseases is unknown, and the specific role played by autoantibodies in the onset of various autoimmune connective tissue diseases is obscure, the Association and frequency of detection of these antibodies, particularly those of the IgG class, ANA Screen ELISA test system, offers an efficient test procedure for the laboratory workup of patients with suspected various connective tissue diseases. The following table summarizes the various autoantibodies noted above with respect to disease Association.
Limitations	1. The ANA ELISA test is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures. 2. Positive ANA may be found in apparently healthy people. It is therefore imperative that the results be interpreted in light of the patient's clinical picture by medical authority. 3. SLE patients undergoing steroid therapy may have a negative test result. 4. Many commonly prescribed drugs may induce ANA. 5. The ANA Screen ELISA test system will not identify the specific type of ANA present in a positive specimen. Positive specimens should be tested for individual autobodies.

Antigen Gene Information

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Gene Name	BTG3 BTG family, member 3 [Homo sapiens]
Official Symbol	BTG3
Synonyms	BTG3; BTG family, member 3; protein BTG3; ANA; tob55; protein Tob5; B-cell translocation gene 3; abundant in neuroepithelium area protein; TOB5; TOFA; TOB55; MGC8928;
GeneID	10950
mRNA Refseq	NM_001130914
Protein Refseq	NP_001124386
MIM	605674
UniProt ID	Q14201
Chromosome Location	21q21.1
Pathway	RNA degradation, organism-specific biosystem; RNA degradation, conserved biosystem;

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