ANA Screen IgG

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Antinuclear antibodies (ELISA)

REF: DS167742 Σ **96**

Intended use
The DiaSino ANA Screen IgG assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro qualitative determination of human autoantibodies of the immunoglobulin class IgG against 10 different antigens (dsDNA, histones, ribosomal P-proteins, nRNP, Sm, SS-A, SS-B, Scl-70, Jo-1 and centromeres) in **Human Serum**, it's used for the diagnosis of Sharp syndrome (MCTD), systemic lupus erythematosus, Sjögren's syndrome, progressive systemic sclerosis, polymatomyositis and dermatomyositis.

Summary[1-6]
Antinuclear antibodies (ANAs, also known as antinuclear factor or ANF) are autoantibodies that bind to contents of the cell nucleus. In normal individuals, the immune system produces antibodies to foreign proteins (antigens) but not to human proteins (autoantigens). In some cases, antibodies to human antigens are produced.
There are many subtypes of ANAs such as anti-Ro antibodies, anti-La antibodies, anti-Sm antibodies, anti-nRNP antibodies, anti-Scl-70 antibodies, anti-dsDNA antibodies, anti-histone antibodies, antibodies to nuclear pore complexes, anti-centromere antibodies and anti-sp100 antibodies. Each of these antibody subtypes binds to different proteins or protein complexes within the nucleus. They are found in many disorders including autoimmunity, cancer and infection, with different prevalences of antibodies depending on the condition. This allows the use of ANAs in the diagnosis of some autoimmune disorders, including systemic lupus erythematosus, Sjögren syndrome, scleroderma, mixed connective tissue disease, polymyositis, dermatomyositis, autoimmune hepatitis and drug induced lupus.
Autoantibody screening is useful in the diagnosis of autoimmune disorders and monitoring levels helps to predict the progression of disease. A positive ANA test is seldom useful if other clinical or laboratory data supporting a diagnosis are not present.

Test principle
Indirect method. Total duration of assay: **70 minutes**
Polystyrene microwell strips pre-coated with a pool of these ten antigens. HEp-2 cells were cultured and lysed, all proteins were extracted from cell lysate.
Patient's serum or plasma sample is added, and during the first incubation step, the specific IgG antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins.
A second set of Mouse Anti-Human IgG antibody conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigens is added, and during the second incubation, they will bind to the captured antibody.
The microwells are washed to remove unbound conjugate. Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for ANA IgG remain colorless.

Reagents
Materials provided
- **Coated Microplate - symbol** ANA IgG PLATE 8 x 12 strips, 96 wells, strips pre-coated with a pool of these ten antigens, including dsDNA, histones, ribosomal P-proteins, nRNP, Sm, SS-A, SS-B, Scl-70, Jo-1 and centromeres, and lysed HEp-2 cells
- **Enzyme Conjugate - symbol** ANA IgG CONJ 1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled with Mouse Anti-Human IgG antibody. Contains 0.1% ProClin300 preservative.
- **Sample Diluent - symbol** ANA IgG DILUT 1 vial, 11 mL, ready to use
- **Positive Control - symbol** ANA IgG PC 1 vial, 0.2 mL.
- **Negative Control - symbol** ANA IgG NC 1 vial, 0.2 mL
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 2 pieces.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 µL with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Storage and stability
- Store at 2-8°C.
- Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples and reagents are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
- **Preparation**: Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- **Adding Sample Diluent:** Add **100 µL** of Sample Diluent into their respective wells except the Blank.
- **Adding Sample**: Add **10 µL** of **Positive control, Negative control,** and **Specimen** into their respective wells except the Blank. Mix by tapping the

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plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
Note: After adding Sample, the reagents in wells turns **Blue** color from **Green.**

- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Adding Conjugate:** Add **100 µL** of **Conjugate** into each well except the Blank.
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add **100 µL** of **Substrate** into each well including the Blank. Incubate the plate at room temperature for **10 minutes** avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produces blue color in Positive control and in positive sample wells.
- **Stopping:** Using a multichannel pipette or manually, add **50 µL** of **Stop Solution** into each well and mix gently. Intensive yellow color develops in Positive control and ANA IgG positive sample wells.
- **Measuring:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**.

Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

Quality control and calculation

- Read the sample's optical density (OD) at 450nm with a micro plate reader.
- Mean negative control OD value ≤ 0.1 and positive control OD value ≥ 0.8, the test is valid, otherwise the test is invalid.
- Cut-Off value (C.O.) = Mean negative control OD value + 0.10 (Calculated by 0.05 when Mean negative control OD value is ≤ 0.05, calculated by actual value when Mean negative control OD value is > 0.05).

Positive Results: Sample O.D value ≥ C.O.
Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that ANA IgG has probably been detected using ANA IgG ELISA. All initially reactive specimens should be retested in duplicates using ANA IgG ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for ANA IgG with ANA IgG ELISA.

Negative Results: Sample OD value < C.O
Specimens giving absorbance less than the Cut-Off value are negative for this assay, which indicates that no ANA IgG has been detected with ANA IgG ELISA, therefore the patient is probably not infected with IgG and the blood does not contain ANA IgG.

Limitations

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

Performance Characteristics
Specificity
A study of 210 individuals was tested with DiaSino ELISA kits and found that the specificity was 97.50%.

Sensitivity
Among 76 ANA IgG confirmed were positive when tested with this ELISA, and 75 samples of which were detected with positive, the sensitivity was 98.68%.

Precision
Intra-assay
CV ≤ 15%
Within-run precision has been determined by using 15 replicates of three specimens: a low positive, medium positive and a high positive.

Inter-assay
CV ≤ 20%
Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, medium positive and a high positive. Three different lots of the ANA IgG ELISA Test Kit have been tested using these specimens over a 5-day period.

Analytical specificity

- Interferences are not observed up to concentrations of 0.1 mg/mL Acetaminophen, 0.2 mg/mL Gentistic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/mL Acetosalisilyc Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin and 1 % Ethanol.
- Rheumatoid factors do not interfere with test .
- Cross-Reactivity are not observed in Syphilis, HBsAg, HCV , HCG positive specimens.

Reference

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Symbols

IVD **In vitro diagnostic medical device**	8°C 2°C **Temperature limit**	 **Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	 **Date of manufacture**	 **Use-by date**	Σ **Contains sufficient for <n> tests**
 **Manufacturer**	 **Do not use if package is damaged and consult instructions for use**		

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DiaSino Laboratories Co., Ltd
No.68 Jingnansi Road National Eco & Tech Development Area
Zhengzhou 450000, P.R. China. info@diasino.com