CE

DiaMetra
care for quality

DCM099-7
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ANA Screen

for routine analysis

Qualitative determination of IgG class antibodies against nuclear antigens in human serum or plasma

RUO

LOT
See external label

2°C 8°C

Σ = 96 tests

REF **DKO099**

1. INTENDED PURPOSE

The ANA Screen assay is a manual *in vitro* device intended for the qualitative determination of IgG antibodies directed against Sm (Smith), RNP/Sm, Scl-70, SS-A (Ro) (52kDa e 60kDa), SS-B (La), Jo1, U1-SmRNP, CENP-B, dsDNA and Histones in human serum or plasma. ANA Screen Kit is intended for research use only.

2. CLINICAL SIGNIFICANCE

Anti-nuclear autoantibodies (ANAs) are a heterogeneous group of autoantibodies that recognise nuclear macromolecules and their complexes; antibodies to certain cytoplasmic proteins might also fall into this category[1-6]. These antibodies commonly occur in the sera of patients with systemic autoimmune rheumatic diseases (SARDs) and can bind to DNA, RNA and proteins, as well as complexes of nucleic acids with proteins that are essential for different intracellular functions such as replication and transcription.

ANAs' targets can be divided into two groups in the context of systemic rheumatic autoimmune diseases: antibodies that recognise DNA, histones and nucleosomes[1,2,5,6]; and those that bind to complexes of RNA with RNA-binding proteins (RBPs) and small nuclear ribonucleoproteins (snRNPs)[1].

Autoantibodies directed to nuclear antigens are serological hallmarks of SARDs and their detection is used by clinicians to support the diagnosis of disorders such as SLE, Sjögren syndrome, systemic sclerosis, polymyositis and mixed connective tissue disease. It is well documented that these are complex disorders and characterised by the presence of a wide spectrum of autoantibodies. Furthermore, it has widely been shown that ANAs are also frequently found in the sera of patients with non-rheumatic diseases and healthy subjects that do not develop a rheumatic disease.

Due to the complexity of SARDs, ANA tests should always be considered together with the evaluation of the patient's history, clinical manifestations and other laboratory tests to diagnose these conditions.

3. PRINCIPLE OF THE METHOD

The ANA Screen test is an indirect enzyme immunometric assay (ELISA) based on the binding of present antibodies with Sm, RNP/Sm, SS-A (Ro), SS-B (La), Scl-70, Jo1, U1-SmRNP, CENP-B, dsDNA and Histones antigens coated on the microplates. Any antibodies present in calibrators, controls or prediluted patient samples bind to the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

An anti-human-IgG horseradish peroxidase conjugate solution recognises IgG class antibodies bound to the immobilised antigens. After a 30 minutes incubation any excess enzyme conjugate, which is not specifically bound is washed away with wash buffer.

A chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the colour development is stopped by adding the stop solution. The solution turns yellow at this point. The level of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. Calibrator (1 vial, 1.2 mL)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum
CAL1 REF **DCE002/09906-0**
2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum
Negative Control REF **DCE045/09901-0**
Positive Control REF **DCE045/09902-0**
3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1M, NaN_3 < 0.1%
REF **DCE053-0**
4. Conjugate (1 vial, 15 mL)
Anti h-IgG conjugated with horseradish peroxidise (HRP), BSA 0.1%, ProClin™ < 0.0015%
REF **DCE002/09902-0**
5. Coated Microplate (1 breakable microplate)
Microplate coated with ANA antigens
REF **DCE002/09903-0**
6. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(avoid any skin contact)*
REF **DCE004-0**
7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF **DCE005-0**
8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M pH 7.4 REF **DCE054-0**

4.2. Materials required but not provided
Distilled water

4.3. Auxiliary materials and instrumentation
Automatic dispenser.
Precision Pipetting DevicesMicroplate reader (450 nm, 620-630 nm)

5. WARNINGS
- This kit is intended for *in vitro* use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or ProClin™ 300 as preservative. Avoid the contact with skin or mucosa.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators and the Controls should be handled in the same manner as potentially infectious material.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover, it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, wash through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

6. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate. This procedure is highly recommended when the kit is processed using analysers which are not equipped with disposable tips.
  For this purpose, DiaMetra supplies a separate decontamination reagent for cleaning needles.

7. REAGENT STORAGE AND STABILITY

Store the kit at 2÷8°C in the dark.
- The kit is stable at 2-8°C until the expiry date stated in the external kit label.
- Once opened, the kit is stable at 2-8°C for 6 months.
- The diluted wash solution is stable for 30 days at 2-8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

8. SAMPLE COLLECTION AND STORAGE
The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples.

Sample Storage	Duration
2-8°C	96 hours
Freeze/thaw cycles	3 cycles

9. PROCEDURE

9.1. Preparation of the Calibrators (C_1)

Calibrators are ready to use; the concentration of the Calibrator is printed on the label.

9.2. Preparation of the Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.
It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

9.3. Preparation of Samples

For determination of ANA antibodies, human serum or plasma are the preferred sample matrixes.
All serum and plasma samples must be prediluted with sample diluent 1:100; for example, 10 µL of sample may be diluted with 990 µL of sample diluent.
Fasting samples are not necessary and no special preparations are required. Collect blood by venepuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Neither Bilirubin nor Haemolysis have significant effect on the procedure.

The Controls are ready to use.

9.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_1), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_1	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate for 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate for 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate for 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

10. QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.

DiaMetra recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[7].

11. CALCULATION OF RESULTS

Determine the mean absorbance for each duplicate sample.

To obtain sample value: divide the mean absorbance of the sample by the absorbance of the Calibrator, then multiply by the concentration of the Calibrator, as shown below:

$$Sample\ Conc. = \frac{OD\ sample}{OD\ C1} \times Conc.C1$$

Reactivity is not connected in a linear way to the amount of antibodies present. Although an increase or a decrease in the concentration of antibodies results in increased or decreased responsiveness, the change is not in proportion (e.g., doubling the concentration of antibodies does not lead to a doubling of responsiveness).

To achieve greater accuracy in the determination of antibodies it is recommended to test serial dilutions of the sample. The final dilution that is positive in the test is the antibody concentration of the patient.

12. METROLOGY AND TRACEABILITY

The ANA Screen assay is traceable to the Antinuclear Antibodies (ANA) Reference Standards by Autoantibody Standardisation Committee (ASC) - Center for Disease Control (CDC) and WHO anti-dsDNA 15/174.

13. INTERPRETATION OF RESULTS

ANA Screen (AU/ mL)	Interpretation
< 25	The sample should be considered negative
≥ 25	The sample should be considered positive

The above index and interpretation should be considered as guidelines only. Positive results should be verified concerning the entire clinical status of the patient. Also, every decision for therapy should be taken on an individual patient basis.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum anti-ANA.

14. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

14.1. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 50 negative and 53 positive samples run on a single reagent lot.

		DKO099		Total
		Positive	Negative	
True state	Positive	53	0	53
	Negative	0	50	50
Total		53	50	103

Diagnostic sensitivity: 100%

Diagnostic specificity: 100%

14.2. Precision

Precision of the ANA Screen kit was determined by performing a complex precision study.

Repeatability: A total of A total of 3 serum samples were assayed using 2 lots of reagents in 10 replicates per sample, by 3 operators, once a day for 5 days. Data from one representative lot is shown below:

Repeatability results from one representative lot:

Sample	n	+'ve results	-'ve results	% +'ve results	% -'ve results
Cut off	150	82	68	54.7	45.3
Negative	150	1	149	0.7	99.3
Positive	150	150	0	100.0	0.0

Reproducibility results for the combined data from two lots is shown below:

Sample	n	+'ve results	-'ve results	% +'ve results	% -'ve results
Cut off	300	163	137	54.3	45.7
Negative	300	3	297	1.0	99.0
Positive	300	300	0	100.0	0.0

14.3. Serum-plasma study

The ANA Screen matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI GP34-A "Validation and Verification of Tubes for Venous and Capillary Blood. Specimen Collection". A total of 20 samples (16 native, 4 contrived) were evaluated.

Sample Type	No. Samples	Results vs Serum	
		False pos	False neg
SST	20	0	0
K2 EDTA	20	0	0
Lithium heparin	20	0	0
Sodium heparin	20	0	0

14.4. Interferents

The following substances do not interfere in the ANA Screen assay when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglyceride	500 mg/dL

15. LIMITATIONS OF USE

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays[8]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

16. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

17. BIBLIOGRAPHY

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18. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

19. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.

The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

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