ANA GENLISA™ ELISA

REF : KBD101

Ver 2.0

IVD

Enzyme Immunoassay for the Qualitative Determination of ANA in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

REF **KBD101** Σ **96 tests**

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Introduction:

The detection of autoantibodies against intracellular antigens called anti-nuclear antibodies (ANA) is important in the diagnosis of systemic autoimmune rheumatic diseases (SARD) such as systemic lupus erythematosus (SLE), Sjögren's syndrome, mixed connective tissue diseases (MCTD), systemic sclerosis (SSc) and idiopathic inflammatory myopathies. ANA testing is usually performed as part of the initial diagnostic workup when suspicion of an underlying autoimmune disorder is high. Furthermore, while the frequency of ANA is highest in patients with SARD, these antibodies are also found in patients with organ-specific autoimmune diseases (autoimmune liver diseases, Hashimoto's thyroiditis), certain infections, cancer, advanced age, and in some healthy individuals. Important associations include anti-dsDNA and anti-Sm antibodies with SLE; anti-Scl70 antibodies with progressive SSc; anti-centromere antibodies with a limited cutaneous form of SSc; and anti-Jo-1 antibodies with myositis. In contrast to the disease specificity of some ANAs, antibodies to SSA/Ro and SSB/La antigens, despite being an important feature of Sjögren's syndrome, also commonly occur in SLE and rheumatoid arthritis. Antibodies to RNP antigens alone are found in MCTD, while RNP antibodies, together with other autoantibodies, may be observed in progressive systemic sclerosis, Sjögren's syndrome, and rheumatoid arthritis.
ANA-IFA using HEp-2 cells is considered the gold standard method for detecting ANA with some cons and pros. With higher sensitivity due to the inclusion of the largest number of antigens in their native conformations, this method encounters variability and lack of reproducibility among different laboratories. However, the ELISA method is more convenient to use and can be easily automated, thus permitting efficient screening of large numbers of patient samples. In addition, ANA ELISA offers equivalent sensitivity and higher specificity compared to ANA-IFA. The ANA Screen IgG kit use HEp-2 cell extract spiked with dsDNA and a combination of purified, recombinant or native proteins including SSA/Ro, SSB/La, sm, RNP, smRNP, centromere B, scl70, and Jo1 associated with common SARD.
The ANA Screen IgG ELISA kit is designed for the qualitative detection of anti-nuclear IgG antibodies that could be applicable as an aid to the diagnosis of certain systemic rheumatic diseases.

Intended Use:

The ANA screen IgG ELISA kit is designed for the qualitative detection of anti-nuclear antibodies (ANA) IgG in human serum or plasma samples. It is intended as an aid to the diagnosis of certain systemic autoimmune rheumatic diseases. This kit is for in vitro diagnostics (IVD) for professional use only.

Principle:

ANA GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay for qualitative determination of IgG antibody present in the human serum and plasma. Nuclear Antigen is pre-coated onto microwells. Samples, Controls are pipetted into microwells and ANA antibody present in sample binds to the antigen coated on the wells. Enzyme Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of ANA present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Nuclear Antigens Microtiter Coated Plate (8x12 wells) - 1 no
2. Sample Diluent– 2 x 50 ml
3. Calibrator – 2 ml
4. Negative Control Serum – 2 ml
5. Positive Control Serum – 2 ml
6. Enzyme Conjugate – 12 ml
7. (10X) Wash Buffer – 50 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 10 ul, 100 ul to 1000 ul
3. Deionized (DI) water
4. Distilled Water
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Test Sample Preparation:

Dilute samples 1:101 with sample diluent (dilute 10 ul of sample with 1000 ul of sample diluent).
Note: Control sera are ready to use and do not need any dilution.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 50 ml of Wash Buffer (10X) to 450 ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add **100 ul Positive Control Serum, Negative Control Serum, Calibrator and diluted Sample** in appropriate wells.

3. Seal the plate and Incubate at room temperature for 60 minutes.

4. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

5. Add **100 ul** of **Enzyme Conjugate** to each well except blank well.

6. Seal the plate and Incubate at room temperature for 30 minutes.

7. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

8. Add **100 ul** of **TMB Substrate** into each well.

9. Incubate at room temperature for 15 minutes.

10. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 30 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Calculate a ratio of the absorbance value of the control or patient sample over the absorbance value of the calibrator according to the following formula:
Index= absorbance value of the control or patient sample/ absorbance value of the calibrator

Interpretation of Results:

Index <0.9	Negative
Index 0.9-1.1	Suspected
Index ≥1.1	Positive

Validity of the Test

The assay is considered valid if:
1. The OD (450 nm) of calibrator higher than 0.17.
2. The OD (450 nm) of positive control higher than 0.8 and for the negative control less than 0.1.

Performance Characteristics:

Comparative Study

The ANA IgG kit was evaluated using the serum samples confirmed by a commercially ANA IgG ELISA assay. The positive serum specimens were collected from rheumatologic patients who attended one of the referral clinical centers. The results are summarized below.

Comparative Study (n= 196)		**Commercial ELISA Kit (ANA IgG ELISA kit)**		
		+	**-**	**Total**
ANA IgG ELISA Kit	**+**	128	8	136
	-	2	58	60
	Total	130	66	196

Test Precision
Intra, as well as Inter-assay precision, carried out by 4 different sera, and the results are shown in tables 1 and 2:

Table 1: Intra-assay

No.	No. of tests*	Mean (S/C)	SD (S/C)	CV %
1	20	0.75	0.048	6.40
2	20	1.54	0.087	5.64
3	20	4.14	0.171	4.13
4	20	8.27	0.405	4.90

Table 1: Inter-assay

No.	No. of tests*	Mean (S/C)	SD (S/C)	CV %
1	20	0.67	0.10	14.93
2	20	1.53	0.14	9.15
3	20	4.61	0.46	9.98
4	20	9.71	1.026	10.57

*Each test was run in duplicate.

Interference

To determine potentially endogenous interfering substances, ANA positive and negative serum samples were spiked with one of the following substances to specified concentration and tested in multiple replicates. No significant interference was found. The results are shown in the table below:

Interferent analyte	The Concentration of the interferent analyte	The value of the specimen before adding the interferent (S/C)	The value of the specimen after adding the interferent (S/C)	The Change of the results (%)
Hemoglobin	1 mg/ml	0.84 7.28 9.71	0.79 7.41 9.56	-5.95 1.76 -1.5
Triglyceride	3000 mg/dL	0.84 7.28 9.71	0.82 7.32 9.73	-2.38 0.55 0.2
Bilirubin	20 ng/dL	0.84 7.28 9.71	0.88 7.35 9.69	4.76 0.96 -0.21

References

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Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

** Under CDSCO Registration, please note*

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Positive Control Serum, Negative Control Serum, Calibrator and diluted Sample** in appropriate wells.
3	Seal the plate and Incubate at room temperature for 60 minutes.
4	Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5	Add **100 ul** of **Enzyme Conjugate** to each well.
6	Seal the plate and Incubate at room temperature for 30 minutes.
7	Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
8	Add **100 ul of TMB Substrate** into each well.
9	**Incubate at room temperature for 15 minutes**.
10	Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 30 minutes stopping the reaction.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
SAMP DIL	**Sample Diluent**
CAL	**Calibrator**
POS CNTRL SERUM	**Positive Control Serum**
NEG CNTRL SERUM	**Negative Control Serum**
ENZY CONJ	**Enzyme Conjugate**
10X WASH BUF	**(10X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**