KRISHGEN PUDGALA
PARTNER FOR PRECISION DIAGNOSTICS

# ANA GENLISA® ELISA

**REF** : KBD101

Ver 3.0

**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

**Krishgen Pudgala LLP** Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

**Introduction:**

The detection of autoantibodies against intracellular antigens called anti-nuclear antibodies (ANA) is essential for diagnosing systemic autoimmune rheumatic diseases (SARD) like systemic lupus erythematosus (SLE), Sjögren's syndrome, and systemic sclerosis. ANA testing is usually part of the initial evaluation when an autoimmune disorder is suspected. While commonly associated with SARD, ANA can also appear in organ-specific autoimmune diseases, infections, cancer, advanced age, and even in some healthy individuals. Antibodies associations include: anti-dsDNA and anti-Sm for systemic lupus erythematosus (SLE); anti-Scl70 for progressive systemic sclerosis (SSc); anti-centromere for limited cutaneous SSc; and anti-Jo-1 for myositis. SSA/Ro and SSB/La antibodies are linked to Sjögren's syndrome but also found in SLE and rheumatoid arthritis. RNP antibodies are characteristic of mixed connective tissue disease (MCTD) but may coexist with other autoantibodies in SSc, Sjögren's syndrome, and rheumatoid arthritis.

The ANA-IFA method using HEp-2 cells is the gold standard for detecting antinuclear antibodies (ANA) due to its high sensitivity from its diverse native antigens. However, it can result in variability across laboratories. In comparison, the ELISA method is more user-friendly and automatable; enabling efficient screening of large samples, and offers similar sensitivity with better specificity than ANA-IFA.

The ANA Screen IgG kit uses HEp-2 cell extract spiked with double-stranded DNA (dsDNA) and proteins related to systemic autoimmune rheumatic diseases (SARD), like SSA/Ro, SSB/La, and Sm. This ELISA kit is designed for the qualitative detection of anti-nuclear IgG antibodies to aid in diagnosing certain systemic rheumatic diseases.

**Intended Use:**

The ANA ELISA Kit is designed for the qualitative detection of anti-nuclear antibodies (ANA) in human serum or plasma samples. It serves as a diagnostic aid for certain systemic autoimmune rheumatic diseases. This kit is intended for in vitro diagnostics (IVD) and is meant for professional use only.

**Principle:**

ANA GENLISA® ELISA is an indirect assay for the qualitative detection of anti-nuclear antibodies (ANA) in human serum and plasma. The microwells are pre-coated with nuclear antigen. Serum or plasma samples are added, allowing ANA antibodies to bind. An enzyme-conjugated antibody is then added, forming an immune complex. After washing to remove non-specific binding, a substrate solution is added, leading to color development proportional to the ANA antibody amount. This reaction is stopped with a stop solution, and absorbance is measured at 450 nm.

**Materials Provided:**

1. Nuclear Antigens Microtiter Coated Plate (96 wells) - 1 no
2. Sample Diluent– 22 ml
3. Calibrator – 1 ml
4. Negative Control – 1 ml
5. Positive Control – 1 ml
6. Enzyme Conjugate – 12 ml
7. (20X) Wash Buffer – 25 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.

**Sample Preparation:**
Prepare 1:21 dilution of test samples, by adding 10 ul of the sample to 200 ul of sample diluent.

**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 25 ml of Wash Buffer (20X) to 475 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, Negative Control, Calibrator and Diluted Sample** in appropriate wells. For the reagent blank, add 10 ul Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.

3. Incubate at room temperature for 20 minutes.

4. Aspirate and wash plate 3 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 20 minutes.

7. Aspirate and wash as per step 4 above.

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

9. Incubate at room temperature for 10 minutes.

10. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 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.

**Cut-Off value (CO) = Calibrator OD x Calibrator Factor (CF).**

Calculate the Antibody (Ab) index of each determination by dividing the O.D. value of each sample by cut-off value.

**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 should be greater than 0.250
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be within the range of 3.2 – 5.9.

**Performance Characteristics:**

**1. Sensitivity and Specificity:**

222 patient sera were tested by this ELISA and a reference ELISA methods. 138 sera were positive and 78 sera were negative by both methods. The agreement between the two methods was 95%. The results are summarized below:

| **Comparative Study (n= 222)** | | **Commercial ELISA Kit (ANA IgG ELISA kit)** | | |
|---|---|---|---|---|
| | | **+** | **-** | **Total** |
| **ANA IgG ELISA Kit** | **+** | 138 | 4 | 142 |
| | **-** | 2 | 78 | 80 |
| | **Total** | 140 | 82 | 222 |

**2. Test Precision:**

**Intra-assay**

| **No.** | **No. of tests*** | **Mean** | **Standard Deviation** | **CV %** |
|---|---|---|---|---|
| 1 | 16 | 1.41 | 0.06 | 4.22 |
| 2 | 16 | 0.82 | 0.02 | 2.6 |
| 3 | 16 | 0.29 | 0.03 | 9.48 |

**Inter-assay**

| **No.** | **No. of tests*** | **Mean** | **Standard Deviation** | **CV %** |
|---|---|---|---|---|
| 1 | 10 | 1.15 | 0.09 | 7.43 |
| 2 | 10 | 0.81 | 0.1 | 11.8 |
| 3 | 10 | 0.29 | 0.03 | 9.48 |

*Each test was run in duplicate.

**Limitations of the test**

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

**References**

1. Tebo AE. Recent approaches to optimize laboratory assessment of antinuclear antibodies. Clin Vaccine Immunol. 2017;24(12):1–28.
2. Pisetsky DS, Bossuyt X, Meroni PL. ANA as an entry criterion for the classification of SLE. Autoimmun Rev. 2019 Dec;18(12):102400.
3. Gniewek RA, Stites DP, McHugh TM, Hilton JF, Nakagawa M. Comparison of antinuclear antibody testing methods: Immunofluorescence assay versus enzyme immunoassay. Clin Diagn Lab Immunol. 1997;4(2):185–8.
4. Elbagir S, Sohrabian A, Elshafie AI, Elagib EM, Mohammed NEA, Nur MAM, Svenungsson E, Gunnarsson I, Rönnelid J. Accumulation of antinuclear associated antibodies in circulating immune complexes are more prominent in SLE patients from Sudan than Sweden. Sci Rep. 2020 Dec 3;10(1):21126.
5. Pisetsky DS. Antinuclear antibody testing-misunderstood or misbegotten? Nat Rev Rheumatol. 2017;13(8):495–502.
6. Choi MY, Cui J, Costenbader K, Rydzewski D, Bernhard L, Schur P. Different indirect immunofluorescence ANA substrate performance in a diagnostic setting of patients with SLE and related disorders: retrospective review and analysis. Lupus Sci Med. 2020 Nov;7(1):e000431.
7. Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Mariën G, Blockmans D, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid-phase assay. Autoimmun Rev. 2011;10(12):801–8.
8. Pisetsky DS, Bossuyt X, Meroni PL. ANA as an entry criterion for the classification of SLE. Autoimmun Rev. 2019;18(12):102400.
9. Tan EM, Smolen JS, McDougal JS, Butcher BT, Conn D, Dawkins R, et al. A critical evaluation of enzyme immunoassays for detection of antinuclear autoantibodies of defined specificities: I. Precision, sensitivity, and specificity. Arthritis Rheum. 1999;42(3):455–64.
10. Nosal RS, Superville SS, Varacallo M. Biochemistry, Antinuclear Antibodies (ANA). 2020 Sep 15. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020 Jan. PMID: 30725756.
11. Ahmad A, Heijke R, Eriksson P, Wirestam L, Kechagias S, Dahle C, Sjöwall C. Autoantibodies associated with primary biliary cholangitis are common among patients with systemic lupus erythematosus even in the absence of elevated liver enzymes. Clin Exp Immunol. 2021 Jan;203(1):22-31.

**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.
- 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.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Pudgala LLP shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Pudgala LLP shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Pudgala LLP be liable for incidental, special, or consequential damages.

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.

Krishgen Pudgala LLP, 2026.

**THANK YOU FOR USING A KRISHGEN PRODUCT!**

**Krishgen Pudgala LLP** Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.

EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

**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, Negative Control, Calibrator and diluted sera** in appropriate wells. For the reagent blank, add 10 ul Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well |
| 3 | Incubate at room temperature for 20 minutes. |
| 4 | Aspirate and wash plate 3 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 20 minutes. |
| 7 | Aspirate and wash as per step 4 above. |
| 8 | Add **100 ul of TMB Substrate** into each well. |
| 9 | **Incubate at room temperature for 10 minutes**. |
| 10 | Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction. |

## SYMBOLS KEY

| | |
|---|---|
| MTP | **Microtiter Plate (12x8 wells)** |
| SAMP DIL | **Sample Diluent** |
| CAL | **Calibrator** |
| POS CNTRL | **Positive Control** |
| NEG CNTRL | **Negative Control** |
| ENZY CONJ | **Enzyme Conjugate** |
| 20X WASH BUF | **(20X) Wash Buffer** |
| SUB TMB | **TMB Substrate** |
| SOLN STOP | **Stop Solution** |
|  | **Consult Instructions for Use** |
| REF | **Catalog Number** |
|  | **Expiration Date** |
|  | **Storage Temperature** |