INSTRUCTION MANUAL

REF 4289

February 15, 2016

ANA 12 LINE

- 20 x 12 determinations -

CE

Line immunoassay for the determination of IgG antibodies to nuclear and cytoplasmic antigens in human serum or plasma

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telephone: +49 (0) 33708 – 9286 – 0
Fax: +49 (0) 33708 – 9286 – 50

www.genericassays.com

INTENDED USE

ANA 12 LINE is used for the separate qualitative determination of auto-antibodies to nuclear and cytoplasmic antigens (dsDNA, nucleosomes, Sm, ribosomes, histones, RNP, SS-A 60 kDa, SS-A 52 kDA, SS-B, Scl-70, CENP-B and Jo-1) in human serum or plasma.

Systemic autoimmune diseases (such as systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Sjögren syndrome, dermatomyositis, mixed connective tissue disease) are characterized by the production of a variety of autoantibodies directed against components of the cell nucleus or plasma.

Although significance and pathological relevance of some auto-antibodies have not completely understood yet, the detection of auto-antibodies plays an important role in the diagnosis of systemic autoimmune diseases.

ANA 12 LINE allows both the detection of autoantibodies to nucleosomes as well as the detection of autoantibodies to extractable nuclear and cytoplasmic antigens.

ANA 12 LINE offers a rapid and handsome opportunity for the determination of the whole autoantibody pattern in systemic autoimmune diseases in one single test. Allowing the analysis of large and small series **ANA 12 LINE** represents an excellent alternative to other techniques

Tan EM: Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989, 44, 93-151

PRINCIPLE of the TEST

ANA 12 LINE is a sensitive line immunoasay assay for the determination of antibodies to dsDNA, nucleosomes, Sm, ribosomes, histones, RNP, SS-A 60 kDa, SS-A 52 kDa, SS-B, Scl-70, CENP-B and Jo-1 in human serum or plasma.

ANA 12 LINE includes 20 numbered test strips containing 12 lines coated with specific antigens: dsDNA, nucleosomes, Sm, P0, histone complex, RNP (A, C, 68 kDa), SS-A 60 kDa, SS-A 52 kDa, SS-B, Scl-70, CENP-B and Jo-1, respectively. Three test lines serve as positive control, conjugate control and negative control.

Patient sera and strips are incubated in the test tray. During the first incubation antibodies of the patient sample bind to the target autoantigens immobilized on the solid-phase of the strips. Following an incubation period of 30 min at room temperature (RT) while shaking unbound serum components are removed by a washing step.

Bound antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Following an incubation period of 30 min at RT while shaking excessive conjugate is separated from the solid- phase immune complexes by an additional washing step.

HRP converts the colourless substrate solution added into a dark blue precipitating product. After 10 - 12 minutes while shaking the reaction is stopped by a washing step.

Strips are dried for at least 30 min. Different patterns of lines become visible defined by the antibodies in the individual serum samples. Strips are interpreted using the pattern template provided in the kit. Results are regarded to be positive if the colouration of the test line is more intense than the colouration of the negative control line.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C. The use of lipemic or hemolytic samples increases the background reaction and can lead to false positive results.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Neat patient samples have to be used and are diluted directly in the incubation tray (1 + 75).***

TEST COMPONENTS for 20 x 12 determinations

A Ag 20	**Test strips** 20 numbered strips 12 test lines coated with specific antigens: dsDNA (purified), nucleosomes (avian), Sm (bovine), P0 (human recombinant), histone complex (purified), RNP (68 kDa, A, C; human recombinant), SS-A (60 kDa, bovine), SS-A 52 kDa (human recomb.), SS-B (human recombinant), Scl-70 (human recombinant), CENP-B (human recombinant), Jo-1 (human recombinant) - Positive control - Conjugate control - Negative control	20 strips
B BUF WASH 10x	**Buffer, 10 fold** sufficient for 450 ml wash buffer (colourless)	45 ml concentrate capped white
C DIL	**Sample diluent** (coloured yellow)	45 ml ready to use capped black
D CONJ	**Conjugate** anti-human-IgG (sheep), conjugated with horse radish peroxidase (coloured red)	45 ml ready to use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide (black bottle)	45 ml ready to use capped blue
F	**Incubation tray** for 10 test strips	2
G	**Interpretation template** for glueing of processed strips	1

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- pipette tips
- multi-channel pipette
- trough for multi-channel pipette
- graduated cylinders
- distilled or de-ionized water
- 2 ml tubes
- horizontal plate shaker
- plastic pincers

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Size and Storage

ANA 12 LINE has been designed for 20 x 12 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the ANA 12 LINE have to be kept at 2- 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

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Preparation before use

Allow all components to reach room temperature prior to use in the assay.

Touch strips with a plastic pincer only.

Prepare a sufficient amount of wash buffer solution by diluting the concentrated buffer 10 times (1 + 9) with deionized or distilled water.
For each strip 13 ml wash solution is required.
For example, dilute 2 ml of the concentrate with 18 ml of distilled water. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

The buffer solution prepared is stable at 2 - 8 °C up to 30 days.

All other components are ready for use and stable until the expiry date.

Avoid exposure of the TMB substrate solution to light.

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ASSAY PROCEDURE

Avoid any time shift during pipeting of reagents or neat samples.

1. Bring all reagents to room temperature (RT) (18-25°C) before use. Mix gently without causing foam.
2. Place the strips (A) with the reactive side up (labels on top) into the respective well. Dispense 1.5 ml of sample diluent (C) into the respective wells.
3. Incubate **5-10 min** at RT (18-25°C) while shaking.
4. Add **20 µl** patient serum or plasma to the respective wells**.**
5. Incubate **30 min** at RT (18-25°C) while shaking.
6. Decant or aspirate, wash each well **three** times **3 min** with **1.5 ml** wash solution (made of B) while shaking. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
7. Add 1.5 ml conjugate (D) to each well
8. Incubate **30 min** at RT (18-25°C) while shaking.
9. Decant or aspirate (see 6.) and wash each well **three** times **3 min** with **1.5 ml** wash solution (made of B) while shaking.
10. Add 1,5 ml of substrate (E) to each well.
11. Incubate **10-12 min** at RT (18-25°C) while shaking.
12. Decant or aspirate (see 6.) and wash each well **once 3 min** with **1.5 ml** wash solution (made of B) or deionised or distilled water to stop the reaction.
13. Dry the strips: Collect the strips from the wells and put onto absorbent paper with the **reactive side upwards**. After approximately **30 min** the strips are to be interpreted using the interpretation template.

EVALUATION of RESULTS

Results should be interpreted only after strips have been dried for at least 30 minutes.

Validation criteria:

Both the **positive and the conjugate controls** must be positive in all cases. The colouration of these lines ensures that the test has been run correctly and the kit components are not degraded. If the positive control or the conjugate control lines shows no colouration the results cannot be interpreted.

The **negative control** demonstrates the extent of non-specific antibody binding of the sample in the test. The colouration of this line serves as sample specific cut-off for positive-negative decision.

The positive **conjugate control** shows that the conjugate works well.

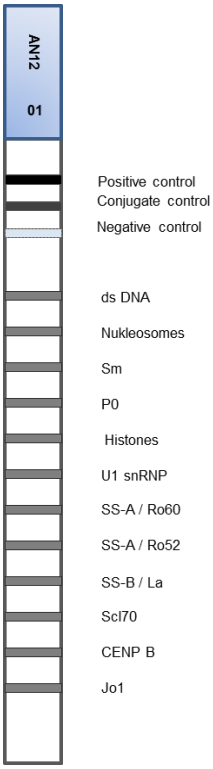
The **test lines** are coated with autoantigens and detect specific antibody binding of the sample in the test. The colour intensity depends on the titer of specific antibody binding in the sample.

Positive result:
A sample is considered to be positive for autoantibodies if the colouration of the test line is more intense than the colouration of the negative control.

The colour intensity of the negative line depends on the test conditions (e.g. incubation times, temperature, washing efficiency) and on the composition of each individual sample.

Negative result:
A sample is considered to be negative for autoantibodies if the colouration of the test line is less intense or equal than the colouration of the negative control.

Test setup



Limitations of the method

Healthy individuals should be tested negative by the ANA 12 LINE. However, ANA positive apparently healthy individuals do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Sensitivity

Relative sensitivity of ANA 12 LINE has been determined by analyzing 53 ANA positive samples of patients suffering from systemic rheumatic diseases in comparison to another Line immunoassay. Agreement of the parameters have been found to:

dsDNA	100%
Nucleosomes	94%
Sm	100%
P0	100%
Histons	87%
RNP	100%
SS-A/Ro60	100%
SS-A/Ro52	100%
SS-B/La	98%
Scl-70	96%
CENP-B	96%
Jo-1	100%

Specificity

Diagnostic specificity was estimated by investigation of a panel of apparently healthy blood donors (n=72) and found with 98%.

INCUBATION SCHEME

ANA 12 LINE (4289)

1.	Bring all reagents and the requested number of strips to room temperature (18-25°C)	
2.	Place the strips with the reactive side upside in the tray and dispense 1.5 ml of Sample diluent (C) into the respective wells	
3.	Incubate while shaking	5-10 min, RT (18-25°C)
4.	Pipette 20 µl patient serum or plasma (final dilution 1 + 75) into each well	
5.	Incubate while shaking	30 min, RT (18-25°C)
6.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
7.	Pipette 1.5 ml conjugate (D) in the respective well	
8.	Incubate while shaking	30 min, RT (18-25°C)
9.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
10.	Pipette 1.5 ml substrate (E)	
11.	Incubate while shaking	10 - 12 minutes, RT (18-25°C)
12.	Decant, wash strips to stop reaction	1 x 3 min with 1.5 ml (made of B) or deionised water
13.	Put the strips **reaction side up** onto absorbent paper to dry. After approximately 30 min the strips are ready to be interpreted.	

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted 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 at 2 - 8 °C prior use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (< 1.0 % w/w) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- 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.