


ANA 12 Line Dot

- 20 x 12 determinations -



Immunodot 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



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INTENDED USE

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

ANA 12 Line Dot 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 Dot represents an excellent alternative to other techniques. Employing a specific negative control non specific colouration depending on the test conditions and sample features are eliminated allowing an easy qualitative interpretation of the results.

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 Dot is a sensitive immunodot 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 Dot includes 20 numbered test strips containing 12 line dots 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. Two test lines serve as function and cut-off controls.

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 45 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. Following an incubation period of 45 min at RT while shaking excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

Horseradish peroxidase 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 by pressing the reactive side onto absorbent paper. 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 dot is more intense than the colouration of the cut-off 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 + 100).*

TEST COMPONENTS for 20 x 12 determinations

A	Dot strips	20 dot strips
Ag	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) - Function control - Cut-off control	
B	Buffer, 5 fold	2 x 35 ml
BUF	sufficient for 350 ml wash and incubation	concentrate
WASH		capped black
	5x	
D	Conjugate	40 ml
CONJ	anti-human-IgG (sheep), conjugated with horse radish peroxidase	ready to use
	G	capped red
E	Substrate	50 ml
SOLN	3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	ready to use
TMB	(black bottle)	capped blue
F	Incubation tray with lid	2
G	Pattern template	1
	(lot specific scan of one positive and one negative dot strip)	

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

Size and Storage

ANA 12 Line Dot 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 Dot 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.

Preparation before use

Take dot strips with a plastic pincer only.

Prepare a sufficient amount of buffer solution by diluting the concentrated buffer 5 times (1 + 4) with deionized or distilled water. For each strip 10.5 ml of buffer solution is required. For example, dilute 15 ml of the concentrate with 60 ml of distilled water.

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

Avoid exposure of the TMB substrate solution to light.

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). Dispense **1.5 ml** of buffer solution (made of B) into the respective wells.
3. Seal plate, incubate **5 min** on an horizontal or rocking shaker.
4. Add **15 µl** neat patient serum to the respective wells.
5. Cover tray and incubate **45 min** while shaking at RT (18-25°C).
6. Decant or aspirate, wash each well **three times 5 min** with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
7. Add **1.5 ml** conjugate (D) to each well.
8. Cover tray, incubate **45 min** at RT (18-25°C) while shaking.
9. Decant or aspirate, wash each well **three times 5 min** with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
10. Add **1.5 ml** of substrate (E) to each well.
11. Cover tray, incubate **10 min** while shaking at RT (18-25°C).
12. Decant or aspirate, wash each well **three times with 1.5 ml** distilled water to stop the reaction.*
13. Collect the strips from the wells and dry the membrane by pressing briefly the reactive side of the strip on the absorbent paper. After approximately **30 min** the strips are interpreted by means of the lot specific pattern and the cut-off line.

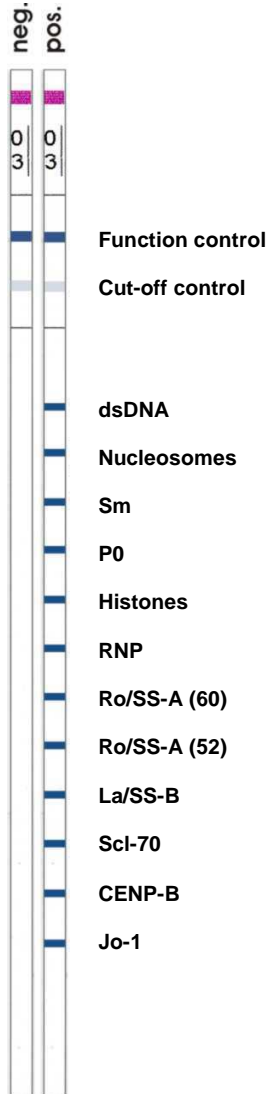
* **Some sera show unspecific background staining of the strips. The reaction has to be stopped quickly by rinsing the strips three times with 1.5 ml aqua dest..**

DATA PROCESSING

Evaluation criteria

Results should be interpreted only if the specific control line is clearly and the cut-off line weakly visible.

The template supplied with the kits shows one lot-specific positive and negative strip each.



Evaluation

Only dried dot strips should be interpreted. The interpretation of the dot strips is based upon the comparison of the developed antigen lines with the pattern template and the cut-off line. The results have to be interpreted as follows.

ANA 12 Line Dot	
positive	Colour intensity of the antigen lines ≥ cut-off line
negative	Colour intensity of the antigen lines < cut-off line

Limits of the method

The in vitro results should always be interpreted in context with the clinical status of the patient. Repeated testing over several weeks is recommended in order to discriminate an active infection from long term persistent antibody titer without clinical implication.

In all immunologic methods bacteriological or fungal contaminations of the kit components and samples as well as cross-reactivity may cause false results.

Insufficient washing or time management may lead to false negative results.

CHARACTERISTIC ASSAY DATA

For the determination of the diagnostic sensitivity and specificity of the ANA 12 Line Dot clinically defined sera of patients as well as sera of healthy blood donors were investigated in ANA 12 Line Dot and compared to the results of another commercial assay. The comparison showed a total agreement of > 95%.

Remarks:

INCUBATION SCHEME

ANA 12 Line Dot (4089)

1.	Bring all reagents and the requested number of strips to RT (18-25°C)
2.	Place the strips with the reactive side upside in the tray and dispense 1.5 ml of buffer solution (made of B) into the respective wells
3.	Seal plate and incubate while shaking 5 min, RT (18-25°C)
4.	Pipette 15 µl neat patient serum
5.	Incubate while shaking 45 min, RT (18-25°C)
6.	Decant, wash strips while shaking 3 x 5 min with 1.5 ml (made of B)
7.	Pipette 1.5 ml conjugate (D)
8.	Incubate while shaking 45 min, RT (18-25°C)
9.	Decant, wash strips while shaking 3 x 5 min with 1.5 ml (made of B)
10.	Pipette 1.5 ml substrate (E)
11.	Incubate while shaking 10 min, RT (18-25°C)
12.	Decant, wash strips with aqua dest. 3 x with 1.5 ml

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 before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservatives. 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.