

Test Instructions

Enzyme Immunoassay for the Detection of Antinuclear and Anti-ENA-Antibodies - Cut Off Test (IMTEC-ANA/ENA-CombiScreen)

Catalogue-No.: TC 70000

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Never mix reagents from different test kit lots.

Store reagents at 2-8°C.

1. Clinical Use

Antinuclear antibodies (ANA) are autoantibodies with different specificity which are directed against antigens of the cell nucleus. In general, ANA can be divided into antibodies directed against extractable nuclear antigens, nonextractable nuclear antigens and cytoplasmatically located antigens.

The detection of ANA and ENA-antibodies is important for diagnosis of collagenosis, especially of systemic lupus erythematosus (SLE) and the mixed connective tissue disease (MCTD) which is strongly associated with it, and also for the diagnosis of other rheumatic diseases.

In SLE patients and MCTD patients ANA are detectable at almost 100 %. From this follows, that the absence of these antibodies practically excludes a diagnosis of these. A posi-

tive detection of ANA is on the other hand no proof of these diseases because ANA are detectable in other diseases, too.

The nomenclature of extractable nuclear antigens and their antibodies is not standardized. They are either named after the first letter of name of the patient in whom the antibody was first detected (Sm, Ro, La, Jo etc.) or after the disease (SS-A, SS-B: Sjogren`s Sndrome, Scl-70: Scleroderma). They are also named after the biochemical structure of the antigen (nRNP: Ribonucleoprotein).

Since new autoantibodies against previously unknown ENAs are periodically discovered, the base of information on the structure of these antigens and the clinical significance of the antibodies directed against them is constantly growing. This is a continuing process, and the discovery of even more new autoantibodies is to be expected. The most important of the already identified ENA antibodies are listed in the table. Because IMTEC coats its ELISA plates with a cell lysate extract enriched with additional antigens, it is possible to detect not only the antibodies mentioned in the table, but also other anti-ENA antibodies not listed there because they are extremely rare or because their antigens have not yet been described. Anti-histone antibodies can also be detected.

type of autoantibody	relevance	specificity	sensitivity
U1-RNP	mixed collagenoses (Sharp-Syndrome/MCTD)	low	95 - 100 %
U2-RNP	mixed collagenoses (Sharp-Syndrome/MCTD)	?	low
Sm	systemic lupus erythematosus	high	15 - 30 %
Ro/SS-A	Sjogren`s-Syndrome	low	90 %
La/SS-B	Sjogren`s-Syndrome	low	85 %
Scl-70	scleroderma	high	70 %
PCNA	systemic lupus erythematosus	high	2 - 5 %
Jo-1	polymyositis/dermatomyositis	high	25 - 35 %
PL-7	polymyositis/dermatomyositis	high	3 - 4 %
PL-12	polymyositis	high	4 - 6 %
Mi-2	dermatomyositis	high	10 - 20 %
Ku	scleroderma-polymyositis-overlap	medium	50 - 60 %
SL	systemic lupus erythematosus	medium	5 - 10 %
CENP-B	CREST-Syndrome	medium	50 - 70 %
PM-Scl	scleroderma-polymyositis-overlap	high	8 - 11 %

2. Principle of the Test

The test is based on the covalent binding of cell nuclei and mixed ENA on different strips of a chemically activated microtiter plate (patent pending) and subsequent binding of the ANA and anti-ENA-antibodies from patient serum. The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG, IgA and IgM. After addition of substrate solution, a color stain develops and its intensity is proportional to the concentration and/or the avidity of the detected antibodies.

3. Material Provided

- microtiter strips (1 x 8), breakable:	color	+ frame
coated with cell nuclei	red	6 pieces
coated with ENA	yellow	6 pieces
- anti-ANA cut off control A, ready to use, (contains sodium azide)		1 ml
- anti-ENA cut off control E, ready to use, (contains sodium azide)		1 ml
- negative control serum, ready to use, (contains sodium azide)		1 vial 1 ml
- positive control serum, ready to use, (contains sodium azide)		1 vial 1 ml
- washing buffer concentrate (10x), (contains thimerosal)		1 bottle 50 ml
- sample buffer concentrate (5x), ready to use, (contains sodium azide)		1 bottle 22 ml
- conjugate buffer, ready to use, (contains thimerosal)		1 bottle 20 ml
- peroxidase conjugate (anti-human IgG), concentrate (100x),		1 vial 200 µl
- peroxidase substrate solution (TMB), ready to use,		1 bottle 12 ml
- stopping solution, H ₂ SO ₄ , ready to use,		1 bottle 12 ml

4. Preparation of Reagents

Allow the kit to reach room temperature!

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2. Preparation of Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute one unit sample buffer concentrate with 4 units distilled water. The ready to use buffer is stable for 6 weeks at 2-8 °C.

4.3. Cut off-controls and Control Sera

The cut off-controls and the control sera are ready to use.

4.4. Preparation of Sera

Use serum samples freshly collected or freeze samples at - 20 °C. Allow the samples to reach room temperature (30 min). Dilute samples 1 : 100 with dilution buffer (10 µl sample to 1 ml buffer).

4.5. Preparation of Conjugate

The amount of conjugate dilution daily required, is to be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution. Dilute conjugate 1:100 with dilution buffer (for 1 plate: 100 µl conjugate with 10 ml buffer, for 2 strips: 20 µl conjugate with 2 ml buffer). Remaining solution should be disposed of.

4.6. Preparation of the Substrate

The TMB-substrate solution is ready to use. Used substrate bottle should be closed carefully. Store substrate solution at 4 - 8 °C protected from light.

4.7. Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2 - 8 °C.

4.8. Stopping Solution

H₂SO₄ (caution!)

5. Test Procedure

- **Pipette 100 µl serum dilution** or (undiluted) standards and control sera into each well, for blanks use ready to use sample diluent buffer instead of serum dilution, seal wells with adhesive foil

- recommended scheme of pipetting

	1	2	3	4	5	6	7	8	9	10	11	12
A	blk						blk					
B	--						--					
C	++						++					
D	ANA cut off						ENA cut off					
E	Pat.1						Pat.1					
F	Pat.2						Pat.2					
G	Pat.3						Pat.3					
H	Pat.4						Pat.4					

ANA ANA ANA ANA ANA ANA ANA ANA ANA ANA ANA ANA ANA
RED YELLOW

- **Incubate for 1 hour** at room temperature (RT)

- **Rinse the wells 3 x** with min. 200 µl washing buffer per well

- **Pipette 100 ml of conjugate dilution** into each well, seal wells with adhesive foil
- **Incubate for 30 minutes at RT**
- **Rinse the wells 3 x** with min. 200 µl washing buffer per well
- **Pipette 100 ml substrate solution** per well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 ml stopping solution** per well.
- **Measure at 450 nm** against a reference wave length > 600 nm within the next 30 min after stopping

6. Interpretation of Results

To prove the functionality of the test, the absorbance of the positive control serum has to be distinctly higher than the absorbance of the cut off control sample. The negative control result has to be lower than the cut off control result.

A patient serum with a measured absorbance that is distinctly higher than the absorbance of the cut off control sample possesses an enhanced level of specific antibodies (positive).

A small enhancement of up to 20% of the absorbance indicates a slightly positive reaction of the patient serum.

If a serum reacts positively in the cut off-test for the corresponding ANA or anti-ENA-antibody should be detected quantitatively using the IMTEC-ANA-Elisa (Cat. No.: TC 60001) or the IMTEC-ENA-Profile-test (Cat. No.: TC 60033: SS-A/Ro, SS-B/La, SmD1, U1-snRNP, Histone, CENP-B, Scl 70, Jo-1).

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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