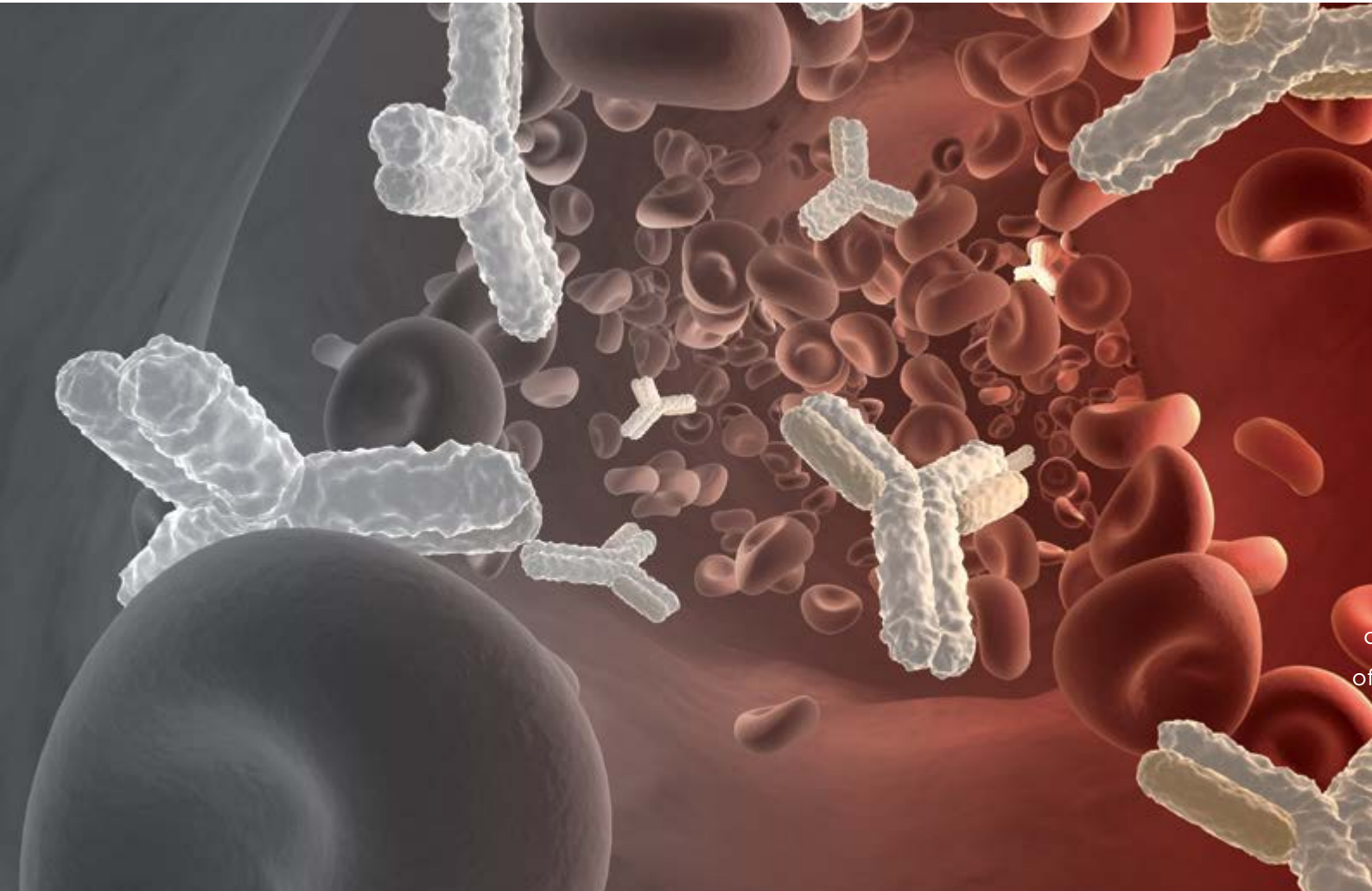


Antinuclear antibodies (ANAs) Antibodies to extractable nuclear antigens (anti-ENAs)



Enzyme immunoassays for the diagnosis
of **systemic autoimmune diseases**

ELISA and **IMMUNOBLOT** kits are optimized and validated for detection of IgG
antinuclear antibodies in human serum and plasma

INTRODUCTION

Determination of antinuclear antibodies is important for diagnosis of systemic autoimmune diseases. These organ non-specific auto-antibodies are directed to intracellular antigens located mainly in the nucleus of the cell. Their detection can indicate the presence of some systemic autoimmunopathologic process - especially: **systemic lupus erythematoses (SLE), Sjögren's syndrome (SS), scleroderma, mixed connective tissue disease (MCTD), systemic sclerosis, polymyositis and dermatomyositis.**

An important group of antinuclear antibodies represent antibodies against ENA (extractable nuclear antigens: SS-A/Ro, SS-B/La, Sm, RNP, Scl-70 and Jo-1). They are mainly ribonucleoproteins and nuclear enzymes.

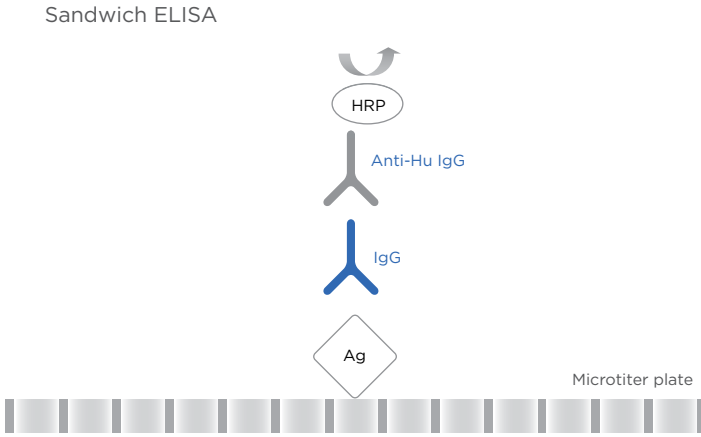
Identification of single auto-antibody specificity is an important tool for differential diagnosis of systemic auto-immune diseases. Antibodies against SS-A/Ro and SS-B/La often occur in patients with SS and SLE. They can be also found in mothers of children with neonatal lupus and congenital heart block. Anti-Sm antibodies represent a highly specific marker and one of diagnostic and classification criteria for SLE. Also anti-RNP antibodies (a part of Sm/RNP complex) are often detected in patients with SLE. Presence of these antibodies is highly specific for MCTD (particularly when anti-Sm antibodies are missing). Detection of anti-Jo-1 antibodies is significant for another group of organ non-specific autoimmune diseases - myositides. Antibodies against antigen Scl-70 and centromere B are typical for diagnosis of systemic sclerosis (particularly its progressive forms).

The group of antinuclear antibodies also includes antibodies against nucleic acids (ssDNA, dsDNA), complexes of nuclear proteins (DNP, RNP) and histones.

ELISA

TEST PRINCIPLE

The assay is based on a sandwich type of ELISA method.



SUMMARY PROTOCOL

Step	Test steps
1	Dilute samples • serum/plasma (1+100)
2	Pipette controls and diluted samples 100 µl • blank = empty well
3	Incubate 30 minutes at 37 °C
4	Aspirate and wash the wells 5 times
5	Add 100 µl Conjugate • blank = empty well
6	Incubate 30 minutes at 37 °C
7	Aspirate and wash the wells 5 times
8	Add 100 µl Substrate (TMB-Complete) • Including blank
9	Incubate 15 minutes at 37 °C
10	Add 100 µl Stopping solution • Including blank
11	Read colour intensity at 450 nm

ADVANTAGES

- ▶ High diagnostic specificity and sensitivity
- ▶ High reproducibility
- ▶ High dynamics of antibody response
- ▶ Ready for automation
- ▶ Customer support

ANTIGENS

EIA ENA screen plus - Mixture of native and recombinant antigens: SS-A/Ro52, SS-A/Ro60, SS-B/La, RNP A, RNP C, RNP 68, SmB, SmD, Scl-70, Jo-1, Centromere B

EIA SS-A - Highly purified native antigen SS-A/Ro60 (60 kDa) and recombinant antigen SS-A/Ro52 (52 kDa)

EIA SS-A/Ro60 - Highly purified native antigen SS-A/Ro60 (60 kDa)

EIA SS-A/Ro52 - Recombinant antigen SS-A/Ro52 (52 kDa)

EIA SS-B - Mixture of native and recombinant antigen SS-B/La

EIA Sm - Mixture of recombinant antigen SmB and native antigen SmD

EIA U1RNP - Mixture of recombinant antigens RNP A, RNP C a RNP 68

EIA Scl-70 - Mixture of native and recombinant antigen Scl-70

EIA Centromere - Mixture of recombinant antigens CENP B a CENP A

EIA Jo-1 - Recombinant antigen Jo-1

CLINICAL APPLICATION

EIA ENA screen plus

- ▶ Screening test for systemic autoimmune diseases by detection of ENA antibodies

EIA ENA (single antigen)

- ▶ Group of tests based on single ENA antigens
- ▶ Differential diagnosis of systemic autoimmune diseases

USER COMFORT

- ▶ Ready-to-use components
- ▶ Colour-coded components
- ▶ Breakable colour-coded microplate strips CUT-OFF included
- ▶ Semiquantitative evaluation of results (Index of Positivity)
- ▶ Easy assay procedure

TEST CHARACTERISTICS

ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA ENA screen plus	96.1%	97.9%
EIA SS-A	95.8%	97.5%
EIA SS-A/Ro60	95.8%	97.5%
EIA SS-A/Ro52	95.8%	97.5%
EIA-SSB	97.9%	97.9%
EIA Sm	97.4%	98.2%
EIA U1RNP	97.7%	98.2%
EIA Scl-70	97.9%	97.9%
EIA Centromere	96.4%	97.9%
EIA Jo-1	95.5%	97.9%

CLINICAL DATA

CLINICAL APPLICATION OF EIA ENA SCREEN PLUS

Blood donors (n = 227) - control group

Negative	3	1%
Positive	227	99%

Patients with autoimmune disease (n = 143)

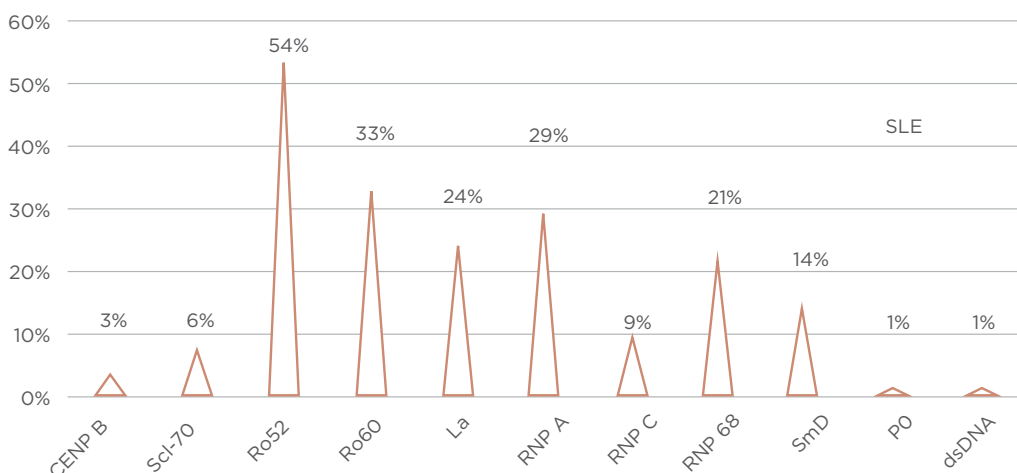
Diagnosis	Tested (n)	Positive (n)	% reaction
SLE	65	63	97%
Sjögren's syndrom	13	12	92%
Sclerodermia	15	14	93%
Dermatomyositis	11	11	100%
Raynaud's syndrome	6	6	100%
Unclear systemic autoimmune disease	33	29	88%

AGREEMENT BETWEEN RESULTS OF EIA ENA SCREEN PLUS AND BLOT ENA PLUS - COMPARISON OF RESULTS FOR GROUP OF POSITIVE SAMPLES AND GROUP OF BLOOD DONORS

Positive samples (n = 380)		Immunoblot		% reaction
		pos	neg	
EIA	pos	286	22	81%
	neg	17	55	19%
% reaction		80%	20%	Correlation 90%

Blood donors (n = 227)		Immunoblot		% reaction
		pos	neg	
EIA	pos	2	1	1%
	neg	7	217	99%
% reaction		4%	96%	Correlation 96%

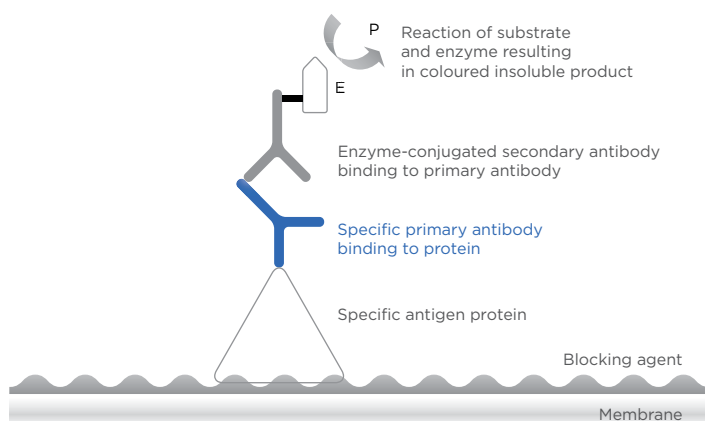
DETECTION OF INDIVIDUAL ANTIGENS FOR GROUP OF PATIENTS WITH SLE - RESULTS OF BLOT-LINE ANA (n = 65)



IMMUNOBLOT

TEST PRINCIPLE

Antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



Anti-Hu IgG: AP-labeled anti-human IgG antibody

SUMMARY PROTOCOL

Step	Test steps
1	Pipette Universal solution 2 ml
2	Strips dipping 10 minutes at room temperature • Shaker
3	Aspirate
4	Dilute samples • serum/plasma 1:51 (30 µl + 1,5 ml)
5	Pipette Controls and diluted samples 1.5 ml
6	Incubate 30 min. at room temperature • Shaker
7	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. • Shaker
8	Pipette Conjugate 1.5 ml
9	Incubate 30 min. at room temperature • Shaker
10	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. • Shaker
11	Pipette Substrate solution (BCIP/NBT) 1.5 ml
12	Incubate 15 min. at room temperature • Shaker
13	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. • Shaker
14	Sticking and evaluation of strips

CLINICAL APPLICATION

BLOT-LINE ENA plus

- ▶ Confirmatory test for EIA ENA screen plus
- ▶ Differential diagnosis of systemic autoimmune diseases by determination of specific ENA antibodies

BLOT-LINE ANA

- ▶ Confirmatory test for EIA ENA screen plus
- ▶ Differential diagnosis of systemic autoimmune diseases by determination of specific ANAs

USER COMFORT

- ▶ Ready-to-use components
- ▶ Colour-coded strips
- ▶ Positive and Negative controls
- ▶ Control of reaction course and CUT-OFF control are present on the strip
- ▶ Interchangeable components
- ▶ Easy assay procedure

TEST CHARACTERISTICS

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE ENA plus	96.6%	97.5%
BLOT-LINE ANA	96.6%	97.4%

ADVANTAGES

- ▶ Identical assay procedure
- ▶ Easy interpretation and reproducibility of results
- ▶ High diagnostic specificity and sensitivity
- ▶ Compatibility with all commercial immunoblot processing systems
- ▶ Customer support

ANTIGENS

BLOT-LINE ANA

recombinant antigens:

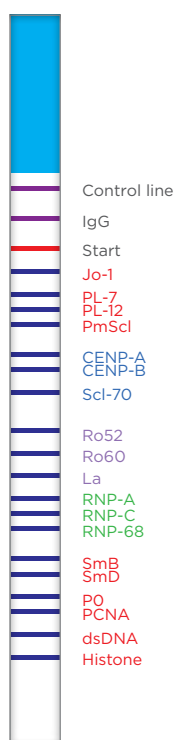
SS-A/Ro52, SS-A/Ro60, SS-B/La, RNP A, RNP C, RNP 68, SmB, SmD, Scl-70, Jo-1, Centromere B, Centromere B, PmScl, PL-7, PL-12, Ribosomal protein PO, PCNA and Histones

BLOT-LINE ENA plus

recombinant antigens:

SS-A/Ro52, SS-A/Ro60, SS-B/La, RNP A, RNP C, RNP 68, SmB, SmD, Scl-70, Jo-1, Centromere B

BLOT-LINE ANA



Polymyositis
Dermatimiositis

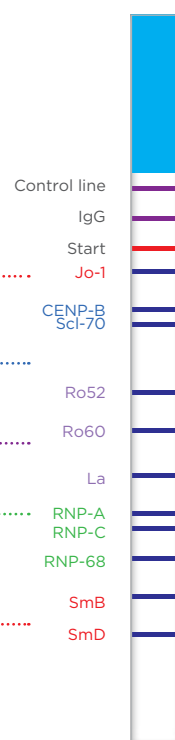
Systemic sclerosis

SS, SLE, Neonatal SLE,
AV block

SLE, MCTD

SLE

BLOT-LINE ENA PLUS



	Antigen	Disease
Jo-1	Histidyl-tRNA synthetase	polymyositis dermatomyositis increased risk of pulmonary fibrosis
PL-7	Threonyl-tRNA synthetase	polymyositis, dermatomyositis antisynthetase syndrome
PL-12	Alanyl-tRNA synthetase	(myositis, interstitial pulmonary fibrosis, arthritis) Raynaud's syndrome
PmScl	11 nuclear polypeptides	polymyositis dermatomyositis systemic sclerosis
CENP B	Centromere B protein	Raynaud's syndrome CREST syndrome
CENP A	Centromere A protein	systemic sclerosis (limited form without the appearance of antigen Scl-70)
Scl-70	DNA Topoisomerase I	systemic sclerosis (specific marker) overlapping syndrome polymyositis - scleroderma (highly specific marker)
Ro52 Ro60	Ro /SS-A (ribonucleoprotein complex)	Sjögren's syndrome (primary, secondary) SLE (dermal forms LE) neonatal LE syndrome
La	La/SS-B (ribonucleoprotein complex)	Sjögren's syndrome (primary) SLE
RNP A RNP C RNP 68	U1-snRNP antigens	MCTD (primarily isolated appearance) SLE Sjögren's syndrome systemic sclerosis (combination with other antibodies)
SmB SmD	Sm antigens (ribonucleoprotein complex)	SLE (highly specific marker)
PO	cytoplasmic ribosomal phosphoprotein	SLE
PCNA	proliferating cell nuclear antigen - processivity factor for DNA polymerase δ in eukaryotic cells	SLE
dsDNA	double-stranded DNA	SLE (40-95%)
Histones	histones 1 to 4, dimer H2A-H2B	SLE (drug induced form of SLE) systemic sclerosis rheumatoid arthritis

ORDERING INFORMATION

ELISA

Cat. No.	Product	No. of Tests
ENA096	EIA ENA screen plus	96
SSA096	EIA SS-A	96
Ro6096	EIA SS-A/Ro60	96
Ro5296	EIA SS-A/Ro52	96
SSB096	EIA SS-B	96
Sm0096	EIA Sm	96
RNP096	EIA U1RNP	96
Scl096	EIA Scl-70	96
CEN096	EIA Centromere	96
Jo1096	EIA Jo-1	96

IMMUNOBLOT

Cat. No.	Product	No. of Tests
ENAL20	BLOT-LINE ENA plus	20
ANAL20	BLOT-LINE ANA	20

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

