

# **Antinuclear antibodies (ANA) Antibodies to extractable nuclear antigens (ENA)**



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

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

## FLYER CONTENTS

Introduction	3
EIA kits	6
▶ For the diagnostics of ENA antibodies	6
▶ For the diagnostics of ENA antibodies – profiles	8
▶ For the diagnostics of dsDNA antibodies	10
Immunoblot kits	12
Microblot-Array – multiplex diagnostics in microtiter plate format	14
Test principle and protocol summary	16
▶ ELISA	16
▶ Immunoblot	17
▶ Microblot-Array	17
List of kits and ordering information	20

## INTRODUCTION

Determination of antinuclear antibodies is important for diagnostics of systemic autoimmune diseases. These organ nonspecific 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 erythematosus (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 autoimmune 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.

Antibodies to double-stranded DNA (anti-dsDNA) fall in the group of antinuclear antibodies. This is a heterogeneous group of antibodies that are directed against various epitopes on a native double-stranded DNA molecule. The antibodies are considered highly specific for systemic lupus erythematosus (SLE).

Examination of anti-nuclear antibodies may also be included the diagnosis process for other autoimmune diseases, such as primary biliary cirrhosis.

The diagnostic process of autoimmune systemic diseases is also enhanced by the detection of anti-DFS70 antibodies, which are common in healthy subjects but rarely occur in patients with systemic autoimmune rheumatic diseases.

## INCIDENCE OF AUTOANTIBODIES IN INDIVIDUAL DISEASES

	Antigen	Antigen origin	Probable association with the disease
Jo-1	Hystidyl tRNA synthetase	Recombinant	ASS, PM, DM
PL-7	Threonyl tRNA synthetase	Recombinant	ASS, PM, DM, Raynaud's phenomenon
PL-12	Alanyl tRNA synthetase	Recombinant	ASS, PM, DM, Raynaud's phenomenon
EJ	Glycyl-tRNA Synthetase	Recombinant	ASS
OJ	Isoleucyl tRNA synthetase	Recombinant	ASS, ILD
KS	Asparaginylyl tRNA synthetase	Recombinant	ILD, PM, DM, ASS
YARS	Tyrosyl tRNA synthetase (Ha)	Recombinant	ASS
ZoA	Phenylalanyl tRNA synthetase	Recombinant	ASS
ZoB		Recombinant	ASS
HMGCR*	3-hydroxy-3methylglutaryl-coenzyme A reductase	Recombinant	IMNM, Statins-induced NM
SAE-1	Small ubiquitin-like modifier activating enzyme	Recombinant	ASS, CDM
SAE-2		Recombinant	ASS, CDM
SRP54	Signal recognition particle	Recombinant	IMNM, PM, DM, ASS
Mi-2	Helicase protein-nuclear transcription	Recombinant	Juvenile DM, DM
TIF1γ	Transcription intermediary factor 1	Recombinant	DM, CDM, Juvenile DM

	Antigen	Antigen origin	Probable association with the disease
MDA5	Melanoma differentiation associated protein 5 (CADM-140)	Recombinant	Amyopathic DM with ILD progression
NXP2	Nuclear matrix protein 2 (p140, MJ)	Recombinant	Juvenile DM
PMScl 100	Human exosome complex	Recombinant	Diffuse SSc, PM/SSc,
PMScl 75		Recombinant	
ScI70	DNA-topoisomerase I	Recombinant	Diffuse SSc, SSc with the risk of the development of pulmonary fibrosis
CENP A	Centromere A	Recombinant	SSc, CREST syndrome
CENP B	Centromere B	Recombinant	SSc, CREST syndrome
POLR3A	RNA polymerase III	Recombinant	Diffuse SSc
NOR90	Nucleolar transcription factor 1 (Ubtfl)	Recombinant	SSc, Raynaud's phenomenon, SLE, SjS
Th/To	Ribonuclease P protein subunit 25 (Rpp25)	Recombinant	SSc with the risk of the development of pulmonary fibrosis
PDGFR-β	Platelet-derived growth factor receptor beta	Recombinant	SSc at risk of developing pulmonary fibrosis, muscular dystrophy and muscle fibrosis
Fibrillarin	U3 RNP - fibrillarin	Recombinant	SSc at risk of developing pulmonary hypertension
Ro52	TRIM21	Recombinant	DM with ILD progression, Raynaud's phenomenon, SLE, neonatal LE, SSc
Ro60	Sjögren's-syndrome-related antigen A (SS-A)	Recombinant	SjS, neonatal LE, SLE
La	Sjögren's-syndrome-related antigen B (SS-B)	Recombinant	SjS, neonatal LE, SLE
RNP A	U1 small nuclear ribonucleoprotein A	Recombinant	SLE, MCTD, Raynaud's phenomenon
RNP 68/70	U1 small nuclear ribonucleoprotein 68/70 kDa	Recombinant	SLE, MCTD, Raynaud's phenomenon
RNP C	U1 small nuclear ribonucleoprotein C	Recombinant	SLE, MCTD, Raynaud's phenomenon
SmB	Smith antigen B	Recombinant	SLE
SmD	Smith antigen D	Recombinant	SLE
PCNA	Proliferating cell nuclear antigen	Recombinant	SLE
PO	Ribosomal protein PO	Recombinant	SLE
Ku	Ku (p70/p80)	Recombinant	SLE, MCTD, PM/SSc
Nucleolin	Nucleolin	Recombinant	SLE
Histons	Histone	Purified native	Detox LE, SLE
Nucleosome	Nucleosome	Purified native	SLE with the risk of the development of lupus nephritis
dsDNA	Double-stranded DNA	Purified native human	SLE
M2	Mitochondrial M2 (AMA-M2)	Recombinant	Primary biliary cirrhosis, SSc with PBC progression
DFS70	Dense fine speckled 70 antigen	Recombinant	Atopic dermatitis, SjS, alone - biomarker for exclusion of SARD

\* Check availability in your country.

ASS	Antisynthetase syndrome
PM	Polymyositis
DM	Dermatomyositis
ILD	Interstitial lung disease
IMNM	Immune-mediated necrotizing myopathy
NM	Necrotizing myopathy
CDM	Cancer-associated myositis
IBM	Inclusion body myositis
SLE	Systemic lupus erythematosus
MCTD	Mixed connective tissue disease
SSc	Systemic sclerosis
SjS	Sjögren's syndrome
PBC	Primary biliary cirrhosis
SARD	Systemic autoimmune rheumatoid disease
IIM	Idiopathic inflammatory myopathy

# EIA kits for the diagnostics of ENA antibodies

Cat. No.	Product	No. of Tests
ENA096	EIA ENA screen plus	96
SSA096	EIA SS-A	96
Ro5296	EIA SS-A/Ro52	96
Ro6096	EIA SS-A/Ro60	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
SK-ENA096	SmartEIA ENA screen plus	96
SK-SSA096	SmartEIA SS-A	96
SK-Ro5296	SmartEIA SS-A/Ro52	96
SK-Ro6096	SmartEIA SS-A/Ro60	96
SK-SSB096	SmartEIA SS-B	96
SK-Sm0096	SmartEIA Sm	96
SK-RNP096	SmartEIA U1RNP	96
SK-Scl096	SmartSmartEIA Scl-70	96
SKCEN096	SmartEIA Centromere	96
SK-Jo1096	SmartEIA Jo-1	96

Extractable nuclear antigens ENA (SS-A/Ro, SS-B/La, Sm, RNP, Scl-70 and Jo-1 represents a group of antinuclear antigens.

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).

## 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

- ▶ Screening test for the detection of antibodies against ENA in systemic connective tissue diseases
- ▶ Tests to detect antibodies against individual ENAs
- ▶ Differential diagnosis of systemic autoimmune diseases

## ADVANTAGES

- ▶ High diagnostic specificity and sensitivity
- ▶ High reproducibility
- ▶ High dynamics of antibody response
- ▶ Total screening time 1,5 hours
- ▶ Ready for automation
- ▶ Customer support

## 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 SS-B	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%

## TYPES OF KITS

SmartEIA kits are designed for automated processing using the Agility® analyser.

### EIA



## 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

## CLINICAL DATA

### CLINICAL APPLICATION OF EIA ENA SCREEN PLUS

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%

Blood donors (n=227) - control group

Negative	3	1%
Positive	227	99%

### SmartEIA





# EIA kits for the diagnostics of ENA antibodies – profiles

Cat. No.	Product	No. of Tests
ENA012	EIA ENA profile	12
ENAp12	EIA ENA profile plus	12
SK-ENA012	SmartEIA ENA profile	12
SK-ENAp12	SmartEIA ENA profile plus	12

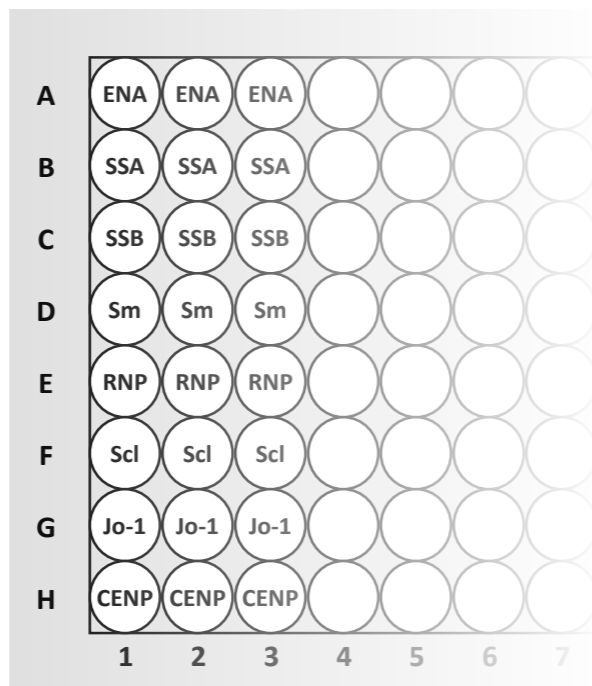
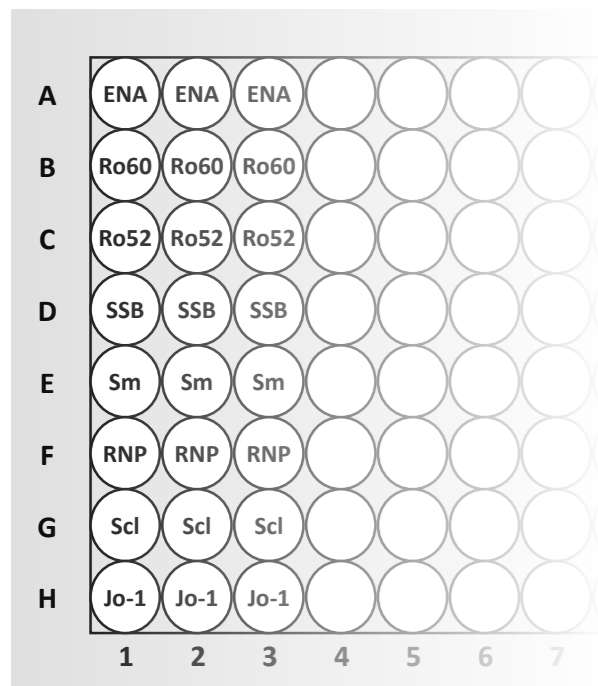
## ANTIGENS

### EIA ENA profile

Raw	Antigens
A	Mixture of native and recombinant antigens Ro52/SS-A, Ro60/SS-A, La/SS-B, Sm, RNP-A, RNP-C, RNP 68, Scl-70 and Jo-1
B	Native antigen Ro60/SS-A
C	Recombinant antigen Ro52/SS-A
D	Recombinant antigen La/SS-B
E	Native antigen Sm
F	Mixture of recombinant antigens RNP-A, RNP-C and RNP 68
G	Mixture of native and recombinant antigen Scl-70
H	Recombinant antigen Jo-1

### EIA ENA profile plus

Raw	Antigens
A	Mixture of native and recombinant antigens Ro52/SS-A, Ro60/SS-A, La/SS-B, Sm, RNP-A, RNP-C, RNP 68, Scl-70, Jo-1 and CENPB
B	Mixture of native antigen Ro60/SS-A and recombinant antigen Ro52/SS-A
C	Recombinant antigen La/SS-B
D	Native antigen Sm
E	Mixture of recombinant antigens RNP-A, RNP-C and RNP 68
F	Mixture of native and recombinant antigen Scl-70
G	Recombinant antigen Jo-1
H	Mixture of recombinant antigens CNEP A and CNEP



## CLINICAL APPLICATION

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

## USER COMFORT

- ▶ Ready-to-use components
- ▶ Colour-coded and interchangeable components
- ▶ Breakable colour-coded microplate strips
- ▶ CUT-OFF, semiquantitative evaluation of results (Index of Positivity - IP)

## TESTS CHARACTERISTICS

ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA ENA profile	97.4%	98.6%
EIA ENA profile plus	95.3%	98.9%

## ADVANTAGES

- ▶ High diagnostic specificity
- ▶ High reproducibility
- ▶ High dynamics of antibody response
- ▶ Short total assay time
- ▶ Customer support

## CLINICAL DATA

### TEST POPULATION

SLE - Systemic lupus erythematoses	79
SSc - Systemic scleroderma	72
DM - Dermatomyositis	23
Blood donors	50
<b>Total</b>	<b>224</b>

### DECLARED NUMBERS OF POSITIVE RESULTS

Tested group	SS-A	Ro60	Ro52	SS-B	Sm	UIRNP	Scl-70	Jo-1	CENP
Systemic lupus erythematoses (n=79)	61	56	44	27	35	32	0	0	3
Systemic scleroderma (n=72)	16	12	15	2	4	4	46	1	18
Dermatomyositis (n=23)	16	7	16	5	3	4	1	6	0
Blood donors (n=50)	0	0	0	0	0	0	0	0	0

### DECLARED NUMBERS OF NEGATIVE RESULTS

Tested group	SS-A	Ro60	Ro52	SS-B	Sm	UIRNP	Scl-70	Jo-1	CENP
Total (n=224)	131	149	149	190	182	184	177	217	203

### RESULTS ACHIEVED ON THE TEST POPULATION

#### EIA ENA profile

Antigen	Sensitivity	Specificity
Ro60	100 %	98 %
Ro52	88 %	99 %
SS-B	82 %	98 %
Sm	93 %	97 %
UIRNP	100 %	98 %
Scl-70	100 %	100 %
Jo-1	86 %	100 %

#### EIA ENA profile plus

Antigen	Sensitivity	Specificity
SS-A	97 %	99 %
SS-B	85 %	98 %
Sm	90 %	97 %
UIRNP	100 %	98 %
Scl-70	100 %	100 %
Jo-1	100 %	100 %
CENP	100 %	98 %

# EIA kits for the diagnostics of dsDNA antibodies

Cat. No.	Product	No. of Tests
DNA096	EIA dsDNA	96
SK-DNA096	SmartEIA dsDNA	96

Antibodies to double-stranded DNA (anti-dsDNA) fall in the group of antinuclear antibodies. This is a heterogeneous group of antibodies that are directed against various epitopes on a native double-stranded DNA molecule. The antibodies are considered highly specific for systemic lupus erythematosus (SLE). Antibodies to dsDNA are present during the active phase of the disease and are associated with lupus nephritis. Their serum concentrations, especially in the IgG class, correlate with disease activity. Their detection is therefore important not only for the diagnosis but also for monitoring the SLE therapy. Presence of anti-dsDNA antibodies is one of the ACR criteria for SLE diagnosis.

Lower titres and low-affinity anti-dsDNA may be present in patients with other systemic diseases / immunopathological conditions (e.g. viral infections), very rarely also in healthy individuals.

Detection of anti-dsDNA via ELISA is one of the sensitive methods. The result should be verified using a specific immunofluorescence test.

## ANTIGENS

- ▶ Purified native human dsDNA

## CLINICAL APPLICATION

- ▶ Sensitive test for detecting antibodies against dsDNA
- ▶ Differential diagnostics of systemic autoimmune diseases
- ▶ High specific marker for SLE

## TEST CHARACTERISTICS

ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA dsDNA	98.0%	98.0%

## USER COMFORT

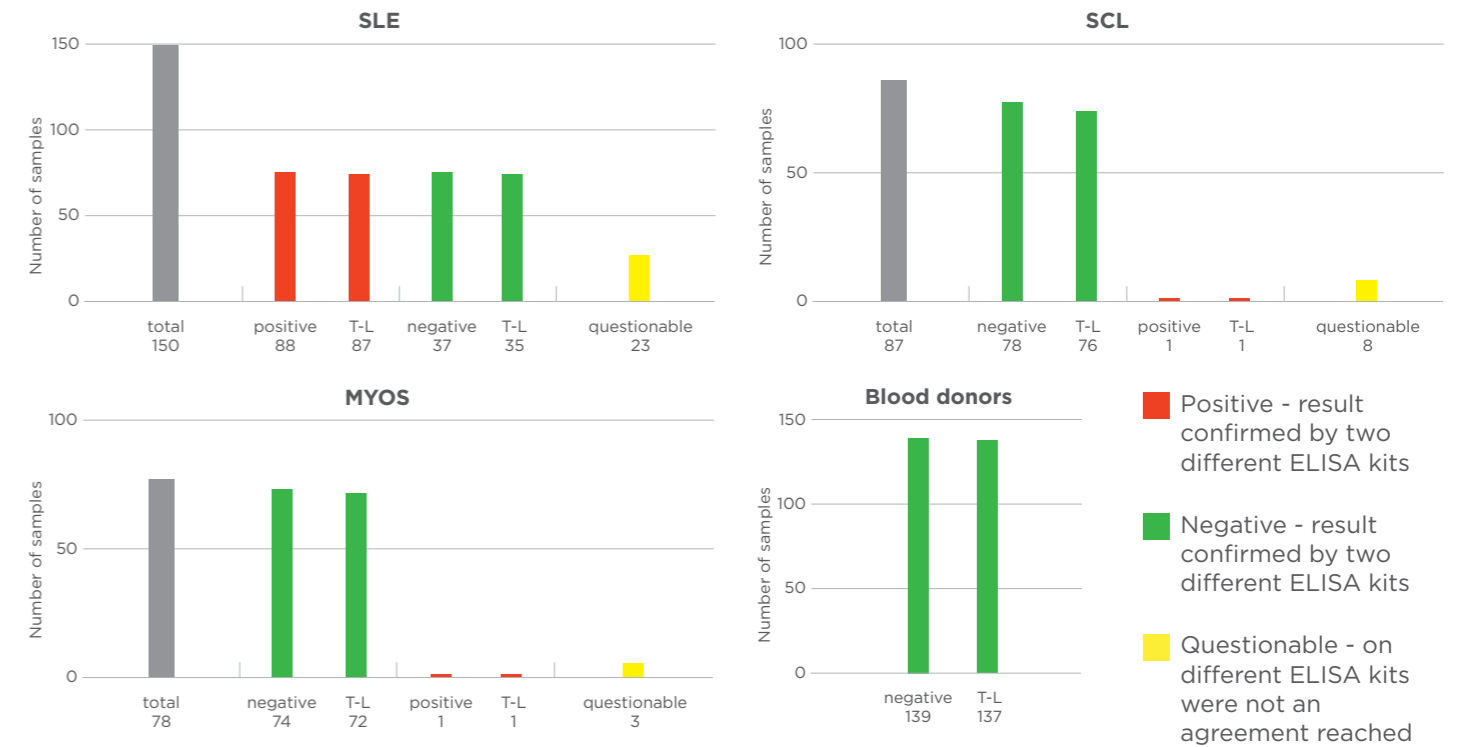
- ▶ Ready-to-use components
- ▶ Colour-coded components
- ▶ Breakable colour-coded microplate strips CUT-OFF included
- ▶ Semiquantitative evaluation of results (Index of Positivity)
- ▶ Calibrators, quantitative evaluation of the results in IU / ml

## ADVANTAGES

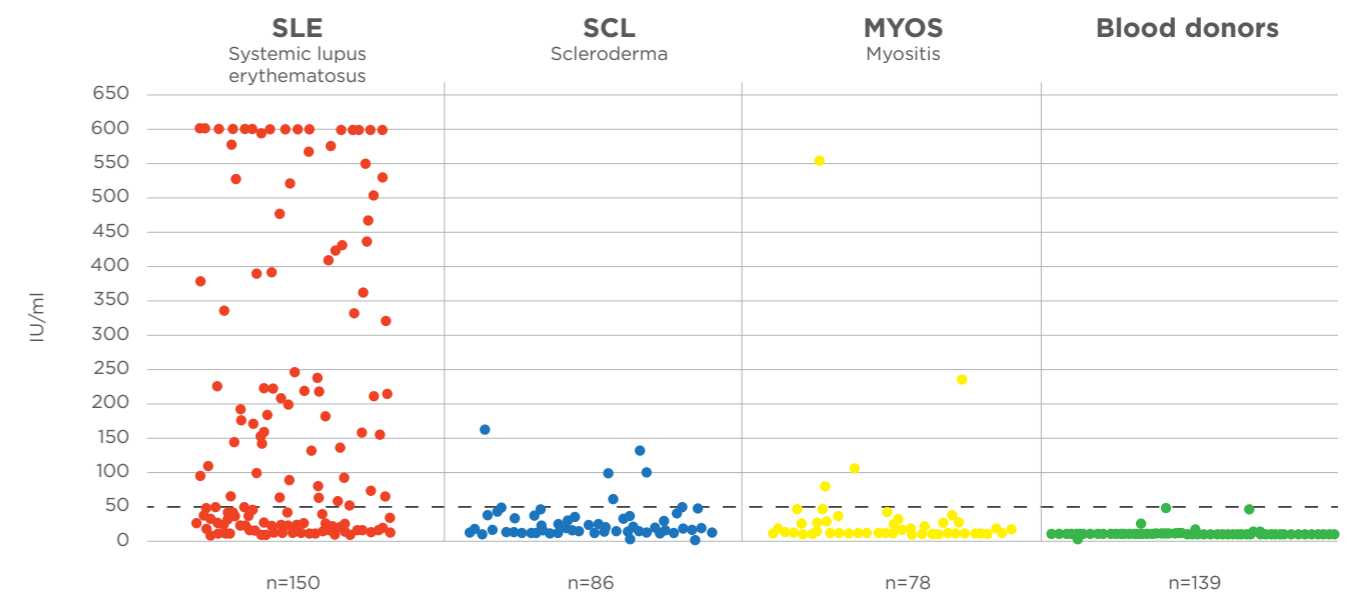
- ▶ High diagnostic specificity and sensitivity
- ▶ High reproducibility
- ▶ High dynamics of antibody response
- ▶ Total screening time 1,5 hours
- ▶ Ready for automation
- ▶ Customer support

## CLINICAL DATA

### RESULTS OF CLINICAL SAMPLES AND CONTROL GROUP TESTING



### DYNAMIC OF THE ANTIBODY RESPONSE



# IMMUNOBLOT kits for the diagnostics of systemic autoimmune diseases

Cat. No.	Product	No. of Tests
ANAL20	BLOT-LINE ANA	20
ENAL20	BLOT-LINE ENA plus <i>on request</i>	20

## 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

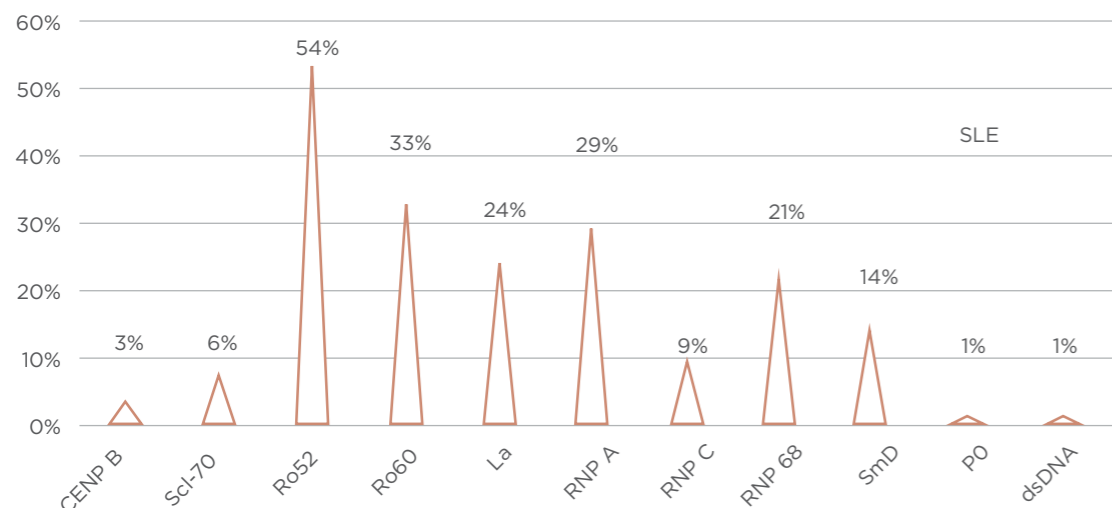
- ▶ Confirmatory test for EIA ENA screen plus
- ▶ Differential diagnosis of systemic autoimmune diseases by determination of specific ANAs

## USER COMFORT

- ▶ Ready-to-use components
- ▶ Colour-coded strips
- ▶ Interchangeable components
- ▶ Positive and Negative controls
- ▶ Control line on the strip
- ▶ Easy assay procedure

## CLINICAL DATA

### DETECTION OF INDIVIDUAL ANTIGENS FOR GROUP OF PATIENTS WITH SLE - RESULTS OF BLOT-LINE ANA



## ADVANTAGES

- ▶ Identical assay procedure
- ▶ Easy interpretation and reproducibility of results
- ▶ High diagnostic specificity and sensitivity
- ▶ Ready for automation
- ▶ Customer support

## TEST CHARACTERISTICS

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

## ANTIGENS

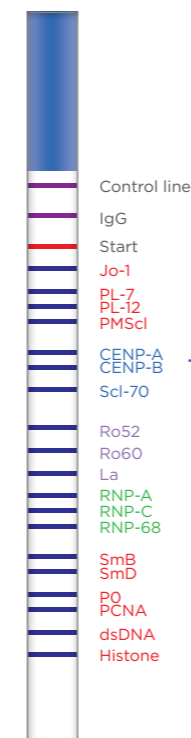
### BLOT-LINE ANA

recombinant antigens:  
Ro52/SS-A, Ro60/SS-A, La/SS-B, RNP-A, RNP-C, RNP 68, SmB, SmD, Scl 70, Jo-1, centromera B, centromera A, PMScl, PL-7, PL-12, ribozomální protein PO, PCNA, dsDNA 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

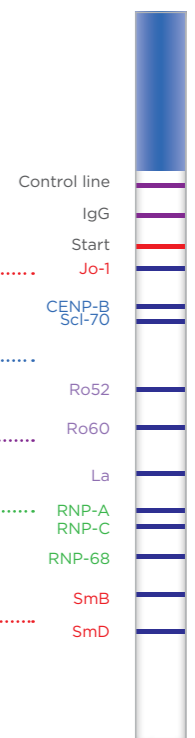
Systemic sclerosis

SS, SLE, Neonatal SLE,  
AV block

SLE, MCTD

SLE

## BLOT-LINE ENA PLUS



## BLOT-LINE KIT



# Microblot-Array - multiplex diagnostics in microtiter plate format for the diagnosis of systemic autoimmune diseases

Cat. No.	Product	No. of Tests
ANAMA96	Microblot-Array ANA	96
ANApMA96	Microblot-Array ANA plus*	96

\*Check availability in your country

Specific recombinant proteins/antigens are spotted onto a nitrocellulose membrane on the bottom of wells of a microtiter plate – providing identical environment as within the classic line-blot strip-assays.

The technology eliminates the bottleneck of traditional blot-processing and opens up the way to high throughput testing.

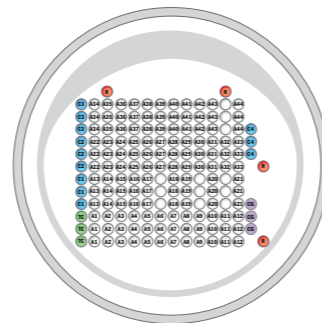
## ADVANTAGES OF TECHNOLOGY

- ▶ Low sample consumption
- ▶ Antigen spotted in triplicate - minimizing statistical verification
- ▶ Fully automatic assay processing and results evaluation
- ▶ Parallel testing of multiple markers simultaneously
- ▶ High sensitivity

## DISTRIBUTION OF ANTIGENS AND CONTROL SPOTS

### Description of antigens Microblot-Array ANA

A1 - Jo-1	A17 - NXP2	A32 - La (SS-B)
A2 - PL-7	A18 - PMScI 100	A33 - PCNA
A3 - PL-12	A19 - PMScI 75	A34 - PO
A4 - EJ	A20 - M2	A35 - SmB
A5 - OJ	A21 - DFS70	A36 - SmD
A6 - KS	A22 - Scl70	A37 - Nucleolin
A7 - YARS (Ha)	A23 - CENP A	A38 - Nucleosome
A8 - ZoA	A24 - CENP B	A39 - Histone
A9 - ZoB	A25 - POLR3A	A40 - RNP A
A10 - HMGCR*	A26 - NOR90	A41 - RNP 68/70
A11 - SAE-1	A27 - Th/To (RPP25)	A42 - RNP C
A12 - SAE-2	A28 - PDGFRβ	A43 - Ku
A13 - SRP54	A29 - Fibrillarin	A44 - dsDNA
A14 - Mi-2	A30 - Ro52	
A15 - TIF1γ	A31 - Ro60 (SS-A)	
A16 - MDA5		



### Description of control spots

■	R - Reference
■	TC - Test control
■	CG - Conjugate control IgG
■	C1 - Calibration 1
■	C2 - Calibration 2
■	C3 - Calibration 3
■	C4 - Calibration 4

## INDIVIDUAL ANTIGENS ARE GROUPED FOR EVALUATION PURPOSES ACCORDING TO THEIR ASSOCIATION WITH THE TYPE OF DISEASE

### ▶ Group 1 (ANA)

Jo-1, PL-7, PL-12, EJ, OJ, KS, YARS, ZoA, ZoB, HMGCR\*, SAE-1, SAE-2, SRP54, Mi-2, TIF1γ, MDA5, NXP2, PMScI 75, PMScI 100, CENP A, CENP B, Scl70, POLR3A, NOR90, PDGFR-β, Fibrillarin, Th/To, Ro52, Ro60, La, SmB, SmD, RNP A, RNP C, RNP 68/70, PO, Ku, Nucleolin, dsDNA, Histone, Nucleosome, PCNA.  
Supplementary antigens: M2, DFS70.

### ▶ Group 2 (Myositis)

Jo-1, PL-7, PL-12, EJ, OJ, KS, YARS, ZoA, ZoB, HMGCR\*, SAE-1, SAE-2, SRP54, Mi-2, TIF1γ, MDA5, NXP2.  
Supplementary antigens: Ro52, PMScI 75, PMScI 100, Ku.

### ▶ Group 3 (Scleroderma)

CENP A, CENP B, Scl70, POLR3A, NOR90, PDGFR-β, Fibrillarin, Th/To, PMScI 75, PMScI 100, RNP A, RNP C, RNP 68/70.  
Supplementary antigens: Ro52, Ku, M2.

### ▶ Group 4 (SLE and other connective tissue diseases)

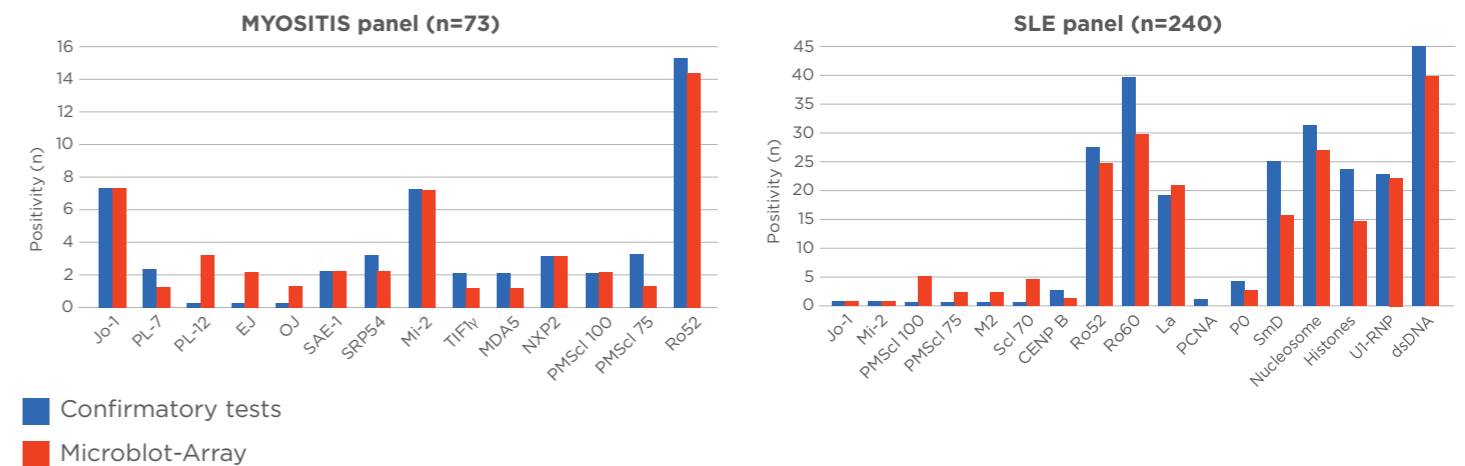
dsDNA, Histone, Nucleosome, PCNA, SmB, SmD, RNP A, RNP C, RNP 68/70, PO, Ku, Nucleolin, Ro52, Ro60, La, NOR90.

\* Check availability in your country.

## RESULTS PROCESSING

- ▶ Overall test evaluation - ANA Positive/Borderline/Negative
- ▶ Evaluation of individual antigens and their association with autoimmune disease type
- ▶ Possibility of evaluation of individual tests - Myopathy/SLE/Scleroderma/Overlap syndromes
- ▶ Test evaluation - qualitative/semi-quantitative/quantitative
- ▶ Results report - simplified/cumulated/detailed

## CONFIRMATORY STUDIES



## MICROBLOT-ARRAY KIT

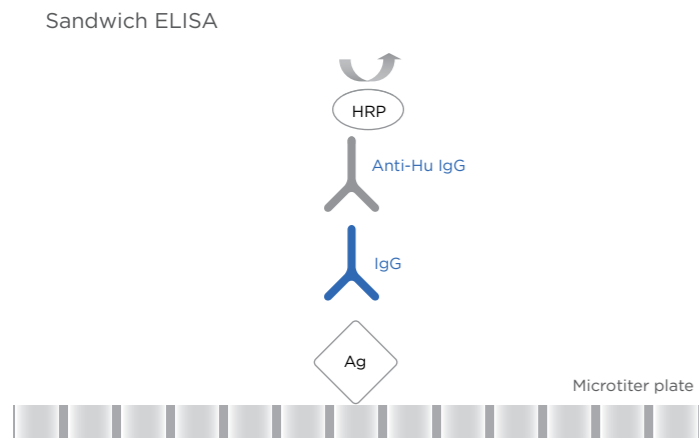




## ELISA

### TEST PRINCIPLE

The assays are based on a sandwich type of ELISA method.



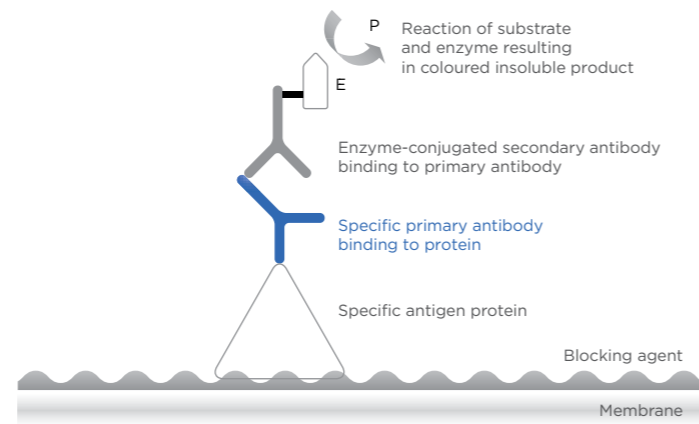
### PROTOCOL SUMMARY

Step	No. Test steps
1	Dilute samples • serum/plasma 1:101 (10 µl + 1 ml)
2	Pipette Controls and diluted samples 100 µl • blank = empty well
3	Incubate 30 min. at 37 °C
4	Aspirate and wash the wells 5 times
5	Add 100 µl Conjugate • blank = empty well
6	Incubate 30 min. at 37 °C
7	Aspirate and wash the wells 5 times
8	Add 100 µl Substrate (TMB-Complete) • Including blank
9	Incubate 15 min. at 37 °C
10	Add 100 µl Stopping solution • Including blank
11	Read colour intensity at 450 nm evaluation of strips

## IMMUNOBLOT

### TEST PRINCIPLE

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

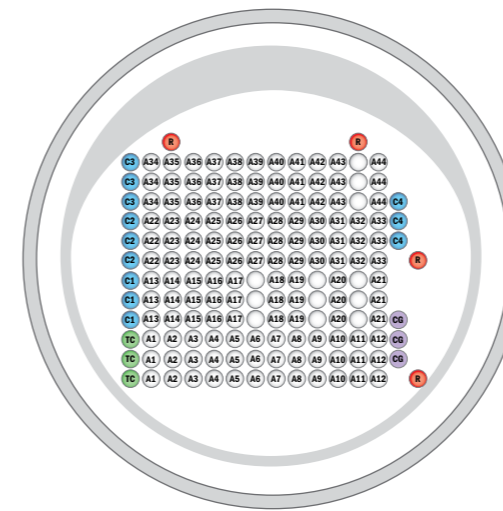


### PROTOCOL SUMMARY

Step	No. Test steps
1	Pipette Universal solution 2 ml
2	Strips soaking 10 min. 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

## MICROBLOT-ARRAY

### DISTRIBUTION OF ANTIGENS AND CONTROL SPOTS



#### Description of antigens

A1 - Jo-1	A17 - NXP2	A32 - La (SS-B)
A2 - PL-7	A18 - PMScl 100	A33 - PCNA
A3 - PL-12	A19 - PMScl 75	A34 - PO
A4 - EJ	A20 - M2	A35 - SmB
A5 - OJ	A21 - DFS70	A36 - SmD
A6 - KS	A22 - Scl70	A37 - Nucleolin
A7 - YARS (Ha)	A23 - CENP A	A38 - Nucleosome
A8 - ZoA	A24 - CENP B	A39 - Histone
A9 - ZoB	A25 - POLR3A	A40 - RNP A
A10 - HMGR*	A26 - NOR90	A41 - RNP 68/70
A11 - SAE-1	A27 - Th/To (RPP25)	A42 - RNP C
A12 - SAE-2	A28 - PDGFRβ	A43 - Ku
A13 - SRP54	A29 - Fibrillarin	A44 - dsDNA
A14 - Mi-2	A30 - Ro52	
A15 - TIF1γ	A31 - Ro60 (SS-A)	
A16 - MDA5		

\* Check availability in your country.

#### Description of control spots

■	R - Reference
■	TC - Test control
■	CG - Conjugate control IgG
■	C1 - Calibration 1
■	C2 - Calibration 2
■	C3 - Calibration 3
■	C4 - Calibration 4

### PROTOCOL SUMMARY

Step	No. Test steps
1	Pipette Universal solution 150 µl
2	Strips soaking 10 min. at room temperature
3	Aspirate
4	Dilute samples • serum/plasma 1:51 (10 µl + 500 µl)
5	Pipette Controls and diluted samples 100 µl
6	Incubate 30 min. at room temperature
7	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
8	Pipette Conjugate 100 µl
9	Incubate 30 min. at room temperature
10	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
11	Pipette Substrate solution (BCIP/NBT) 100 µl
12	Incubate 15 min. at room temperature
13	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
14	Dry and evaluate strips



## LIST OF KITS AND ORDERING INFORMATION

### ELISA

Cat. No.	Products	No. of Tests
ENA096	EIA ENA screen plus	96
ENA012	EIA ENA profile	12
ENAp12	EIA ENA profile plus	12
SSA096	EIA SS-A	96
Ro5296	EIA SS-A/Ro52	96
Ro6096	EIA SS-A/Ro60	96
SSB096	EIA SS-B	96
Sm0096	EIA Sm	96
RNPO96	EIA U1RNP	96
Scl096	EIA Scl-70	96
CEN096	EIA Centromere	96
Jo1096	EIA Jo-1	96
DNA096	EIA dsDNA	96
SK-ENA096	SmartEIA ENA screen plus	96
SK-ENA012	SmartEIA ENA profile	12
SK-ENAp12	SmartEIA ENA profile plus	12
SK-SSA096	SmartEIA SS-A	96
SK-Ro5296	SmartEIA SS-A/Ro52	96
SK-Ro6096	SmartEIA SS-A/Ro60	96
SK-SSB096	SmartEIA SS-B	96
SK-Sm0096	SmartEIA Sm	96
SK-RNPO96	SmartEIA U1RNP	96
SK-Scl096	SmartSmartEIA Scl-70	96
SK-CEN096	SmartEIA Centromere	96
SK-Jo1096	SmartEIA Jo-1	96
SK-DNA096	SmartEIA dsDNA	96

SmartEIA kits are designed for automated processing using the Agility® analyser.

### IMMUNOBLOT

Cat. No.	Products	No. of Tests
ANAL20	BLOT-LINE ANA	20
ENAL20	BLOT-LINE ENA plus <i>on request</i>	20

### MICROBLOT-ARRAY

Cat. No.	Products	No. of Tests
ANAMA96	Microblot-Array ANA	96
ANApMA96	Microblot-Array ANA plus*	96

\*Check availability in your country

## CONTACT

### TestLine Clinical Diagnostics s.r.o.

Krizikova 68, 612 00 Brno, Czech republic

Tel.: +420 549 121 203

Fax: +420 541 243 390

E-mail: sales@testlinecd.com

[www.testlinecd.com](http://www.testlinecd.com)

V01/2019



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

