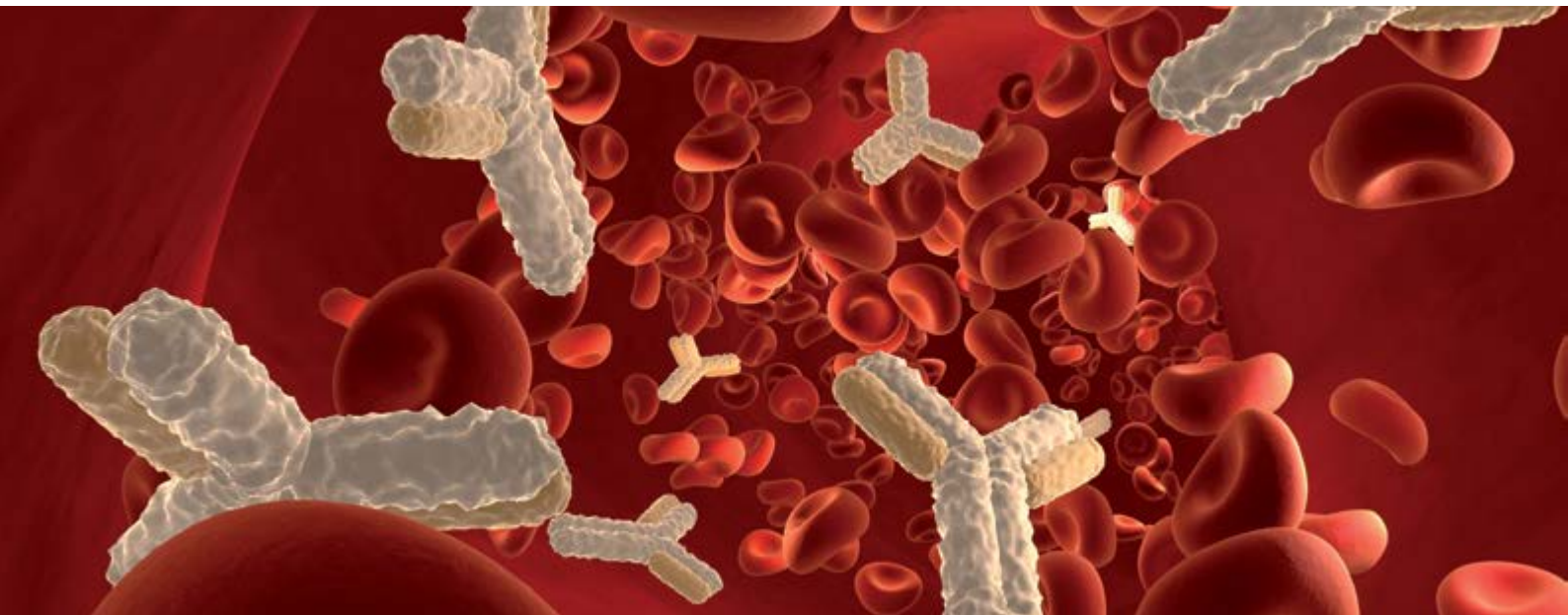# ENZYME IMMUNOASSAYS FOR THE DIAGNOSIS OF SYSTEMIC AUTOIMMUNE DISEASES

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

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

TestLine
Clinical
Diagnostics

Determination of antinuclear antibodies is important for diagnosis of systemic auto-immune 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 erythematodes (SLE), Sjögren's syndrome (SS), sclerodermia, 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-1antibodies is significant for another group of organ non-specific auto-immune 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.
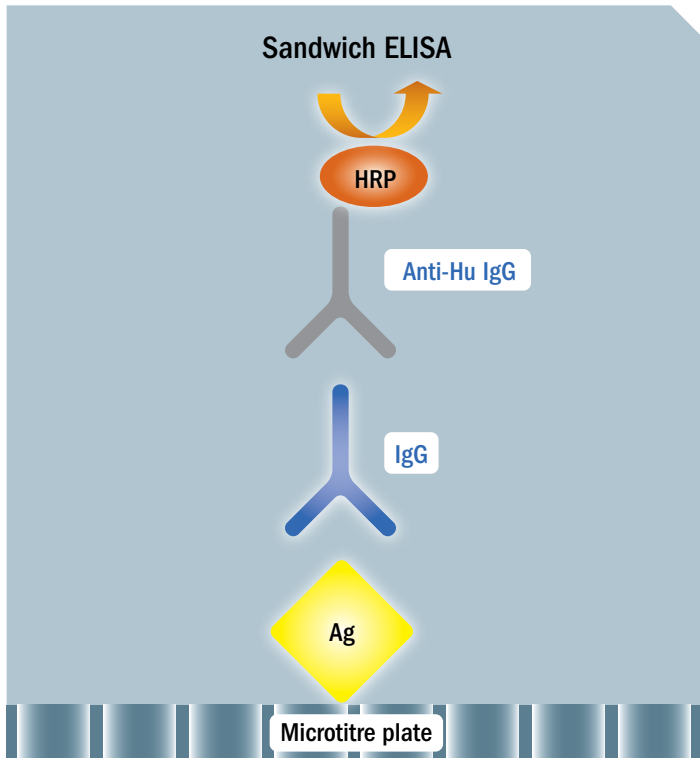
Clinical Application

▶ **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
                                 – Diferential diagnosis of systemic autoimmune diseases

▶ **BLOT-LINE ENA plus**   – Confirmatory test for EIA ENA screen plus
                                 – Diferential diagnosis of systemic autoimmune diseases by determination of specific ENA antibodies

▶ **BLOT-LINE ANA**   – Confirmatory test for EIA ENA screen plus
                                 – Diferential diagnosis of systemic autoimmune diseases by determination of specific ANAs
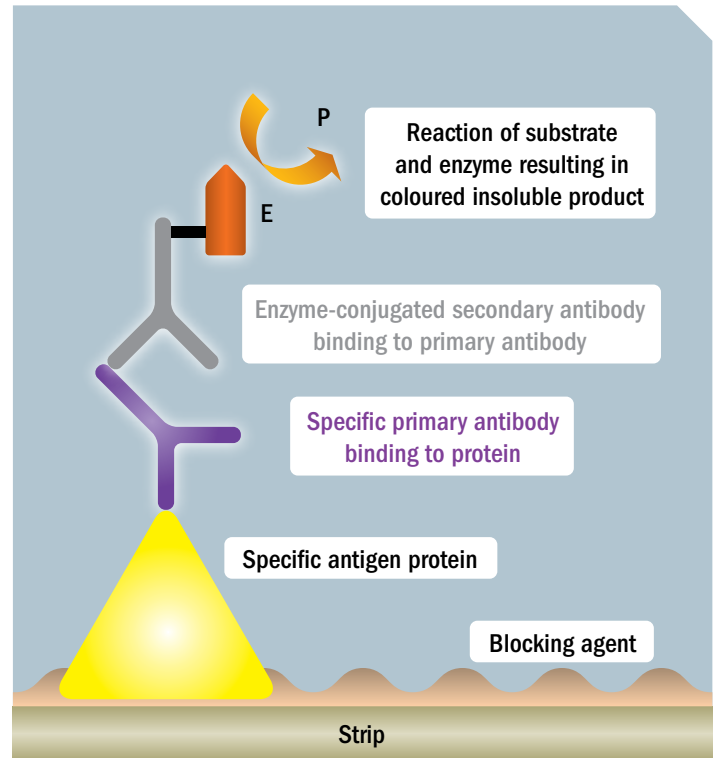
ELISA

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

Sandwich ELISA

HRP

Anti-Hu IgG

IgG

Ag

Microtitre plate

IMMUNOBLOT

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

P

E

Reaction of substrate and enzyme resulting in coloured insoluble product

Enzyme-conjugated secondary antibody binding to primary antibody

Specific primary antibody binding to protein

Specific antigen protein

Blocking agent

Strip

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

ELISA

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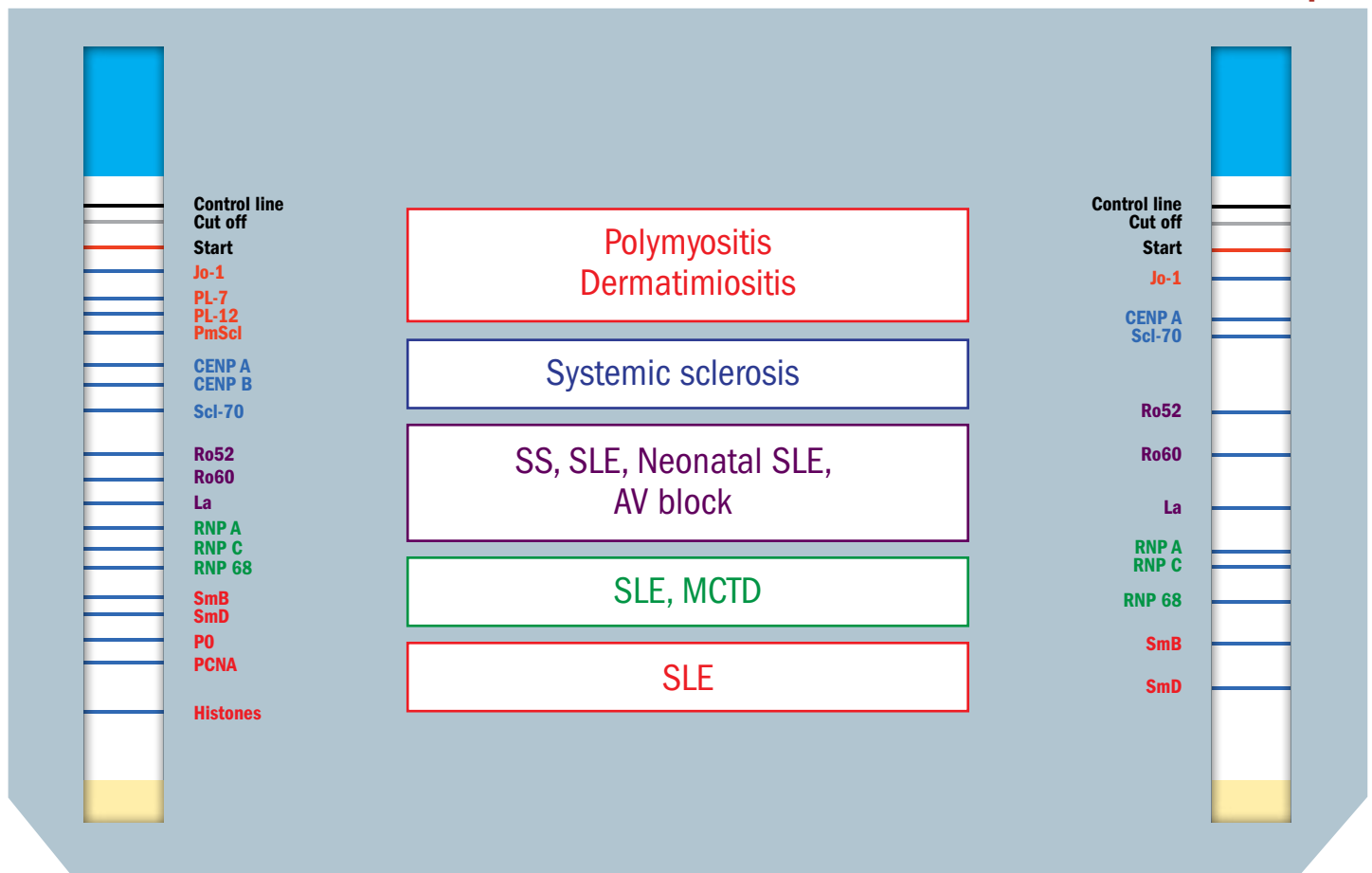- Easy assay procedure

IMMUNOBLOT

- 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

▶ **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

▶ **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** – 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 ANA

BLOT-LINE ENA plus

BLOT-LINE ANA	Disease association	BLOT-LINE ENA plus
Control line / Cut off / Start		Control line / Cut off / Start
Jo-1	**Polymyositis Dermatimiositis**	Jo-1
PL-7 / PL-12 / PmScl		
CENP A / CENP B	**Systemic sclerosis**	CENP A / Scl-70
Scl-70		
Ro52 / Ro60 / La	**SS, SLE, Neonatal SLE, AV block**	Ro52 / Ro60 / La
RNP A / RNP C / RNP 68	**SLE, MCTD**	RNP A / RNP C / RNP 68
SmB / SmD / P0 / PCNA	**SLE**	SmB / SmD
Histones		

	Antigen	Disease
Jo-1	Histidyl-tRNA synthetase	polymyositis dermatomyositis increased risk of pulmonary fibrosis
PL-7 **PL-12**	Threonyl-tRNA synthetase Alanyl-tRNA synthetase	polymyositis dermatomyositis antisynthetase syndrome (myositis, interstitial pulmonary fibrosis, arthritis) Raynaud's syndrome
PmScl	11 nuclear polypeptides	polymyositis dermatomyositis systemic sclerosis
CENP B **CENP A**	Centromere B protein Centromere A protein	Raynaud's syndrome CREST syndrome systemic sclerosis (limited form without the appearance of antigen Scl-70)
Scl-70	DNA Topoisomerase I	systemic sclerosis (specific marker) overlapping syndrome polymyositis – sclerodermia (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 syndrom systemic sclerosis (combination with other antibodies)
SmB **SmD**	Sm antigens (ribonucleoprotein complex)	SLE (highly specific marker)
P0	cytoplasmic ribosomal phosphoprotein	SLE
PCNA	proliferating cell nuclear antigen - processivity factor for DNA polymerase ∂ in eukaryotic cells	SLE
Histones	histones 1 to 4, dimer H2A-H2B	SLE (drug induced form of SLE) systemic sclerosis rheumatoid arthritis

Protocol Summary

ELISA

Step No.		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

IMMUNOBLOT

Step No.		Test steps
1		Pipette Universal solution 2 ml
2		Strips dipping 10 minutes at room temperature Shaker
3		Aspirate
4		Dilute samples serum/plasma (30 µl+1.5 ml)
5		Pipette Controls and diluted samples 1.5 ml
6		Incubate 30 minutes at room temperature Shaker
7		Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 minutes Shaker
8		Pipette Conjugate 1.5 ml
9		Incubate 30 minutes at room temperature Shaker
10		Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 minutes Shaker
11		Pipette Substrate solution (BCIP/NBT) 1.5 ml
12		Incubate 15 minutes at room temperature Shaker
13		Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 minutes Shaker
14		Sticking and evaluation of strips

Advantages

ELISA

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

Immunoblot

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

Test Characteristics

Diagnostic Kit	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%
BLOT-LINE ENA plus	96.6%	97.5%
BLOT-LINE ANA	96.6%	97.4%

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
		poz	neg	
EIA	poz	286	22	81%
	neg	17	55	19%
% reaction		80%	20%	**Correlation 90%**

Blood donors (n = 227)		Immunoblot		% reaction
		poz	neg	
EIA	poz	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)

SLE

Antigen	%
CENP B	3%
Scl-70	6%
Ro52	54%
Ro60	33%
La	24%
RNP A	29%
RNP C	9%
RNP 68	21%
SmD	14%
P0	1%
dsDNA	1%
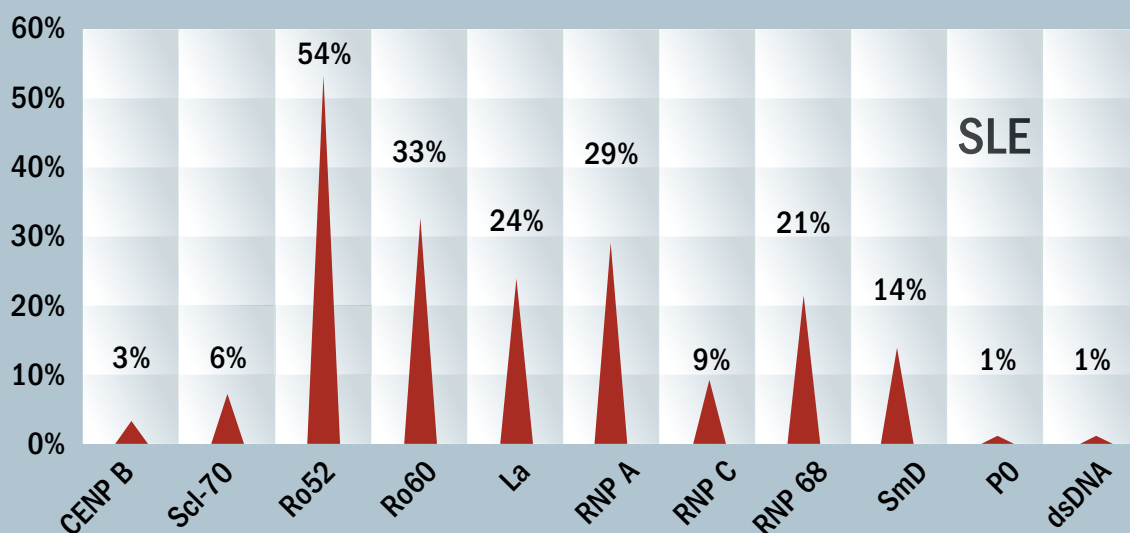
Ordering Information

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

Contact

TestLine Clinical Diagnostics Ltd.
Krizikova 68
612 00 Brno, Czech Republic
Tel.: +420 549 121 209 (218)
Fax: +420 541 243 390
E-mail: trade@testlinecd.com

www.testlinecd.com

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

TestLine
Clinical
Diagnostics