IMTEC-ENA PROFILE

ENA Profile
ELISA for the Detection of Anti-ENA Antibodies (IgG)

Package Size

REF	ITC60033	8x12 Tests	Complete Testkit
IVD			

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

DIL DB14, WASH 20x WB03, SUB TMB ELISA and STOP STOP ELISA may be interchanged between lots and test kits that share the same reagent designation.

All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits.

Store reagents at 2...8°C.

Intended Use

IMTEC-ENA Profile is an indirect solid-phase enzyme immunoassay (ELISA) for the qualitative measurement of IgG class autoantibodies against extractable nuclear antigens in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of connective tissue diseases.

Antibodies to extractable nuclear antigens are characteristic for some rheumatic diseases and can contribute to the diagnosis and prognosis of systemic lupus erythematosus (SLE), Sjögren's syndrome, mixed-connective tissue disease (MCTD), scleroderma, poly- and dermatomyositis and CREST syndrome.

The IMTEC-ENA Profile allows for the simultaneous detection of SS-A/Ro-, SS-B/La-, SmD1-, U1-snRNP-, Scl70-, Jo1-, Histone- and Centromere B-specific autoantibodies.

SS-A / Ro	Antigen: SS-A 60, SS-A 52 Sjögren's syndrome (~90 %), SLE (50 %)
SS-B / La	Antigen: SS-B Sjögren's syndrome (85 %)
SmD1	Antigen: SmD1 peptide SLE (70 %)
U1-snRNP	Antigen: RNP-A, RNP-C, RNP 68 kD Mixed Connective Tissue Disease (100 %)
Scl70	Antigen: Topoisomerase I Systemic Sclerosis (70 %)
Jo1	Antigen: Histidyl-tRNA-synthetase Polymyositis / Dermatomyositis (25 – 30 %)
Histone	Antigen: Histone 2A, 2B, 3, 4 Drug-induced SLE (95 %), SLE (20 – 50 %)
Centromere B	Antigen: Centromere-associated protein B (80 kD) CREST syndrome (**C**alcinosis, **R**aynaud phenomenon, **E**sophageal dysmotility, **S**clerodactyly and **T**elangiectasia)

Principle

The test is based on the absorptive immobilisation of extractable nuclear antigens (native, recombinant and peptidic resp.) to microtiter strips and subsequent binding of the ENA-antibodies. The bound antibodies are detected with a peroxidase-labelled secondary antibody that is directed against human IgG. After addition of substrate solution, a colour appears which intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

Reagents and Contents

MTP	12	**Microtiter Strips** (in 1 strip holder) 8-well snap-off strips, ready for use 12 cavities each coated with: SS-A/Ro (A_{1-12}), SS-B/La (B_{1-12}), SmD1 (C_{1-12}), U1-snRNP (D_{1-12}), Histone (E_{1-12}), CENP-B (F_{1-12}), Scl70 (G_{1-12}) and Jo1 (H_{1-12})
CAL	3 ml	**Calibrator Control** (white cap)
NC	3 ml	**Negative Control Serum** (green cap), human, ready for use
WASH 20x WB03	50 ml	**Washing Buffer** (black cap) Concentrate (20x) for 1 l TRIS buffer pH 6.9 ± 0.2
DIL DB14	100 ml	**Dilution Buffer** (blue cap) ready for use Phosphate buffer pH 7.3 ± 0.2
CON	15 ml	**Conjugate Solution** (white cap) anti-human-IgG HRP conjugate, ready for use
SUB TMB ELISA	15 ml	**TMB solution** (black cap) ready for use, pH 3.7 ± 0.2 colourless to bluish 3,3', 5,5'-tetramethylbenzidin 1.2 mmol/l Hydrogen peroxide 3 mmol/l
STOP STOP ELISA	15 ml	**Stop Solution** (red cap) Sulphuric acid, ready for use 0.5 mol/l
	1	**Adhesive Strip**

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

Reagent Preparation

Attention!

Allow the testkit and all its components to reach room temperature before use! Used bottles should be closed carefully and stored at 2...8°C. Store SUB protected from light.

Do not use polystyrene vessels for handling of CON.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part WASH 20x with 19 parts distilled water. WASH is stable for 6 weeks stored at 2...8°C.

Specimen

Patient sera

Use samples freshly collected or freeze samples at –20°C. **Freeze and thaw once only**. Do not use serum samples inactivated by heat treatment at 56°C.

Allow the samples to reach room temperature (30 min.).

Dilute sera 1:101 with DIL (add 10 µl serum to 1 ml DIL).

Procedure

- **Pipette 8x 100 µl** of diluted patient serum, CAL and optionally NC, into MTP, seal MTP with adhesive strip.
- recommended scheme of pipetting:

	1	2	3	...	12	
A	NC	CAL	pat. 1		pat. 10	SS-A/Ro
B	NC	CAL	pat. 1		pat. 10	SS-B/La
C	NC	CAL	pat. 1		pat. 10	SmD1
D	NC	CAL	pat. 1		pat. 10	U1-snRNP
E	NC	CAL	pat. 1		pat. 10	Histone
F	NC	CAL	pat. 1		pat. 10	CENP-B
G	NC	CAL	pat. 1		pat. 10	Scl70
H	NC	CAL	pat. 1		pat. 10	Jo1

- Incubate for **1 hour** at RT.
- **Discard** the solution from MTP. **Wash** MTP **3 times** using 300 µl WASH per well.

- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µl** CON and seal MTP with adhesive strip.
- Incubate **for 30 min.** at RT.
- **Discard** the solution from MTP. **Wash** MTP **3 times** using 300 µl WASH per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µl** SUB and incubate for **10 min.**. At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 min..
- **Add 100 µl** STOP per well.
- **Read absorbance values at 450 nm** within the next 10 min. after stopping. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended.

Automation

The IMTEC-ENA Profile ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated prior to diagnostic use.

Validation of the Test

The test results are valid provided that the following criteria are met:

- CAL does not fall below an absorbance value of 0.3.
- CAL does not exceed an absorbance value of 3.2
- Optionally: CAL > NC

Interpretation of Results

Interpretation of results can be made by comparing the recalculated absorbances of CAL and of the samples. For each antigen the Cut-Off absorbance should be calculated according to the following equation:

$OD_{Cut\text{-}Off}$ = OD CAL x Specific Factor (see Certificate of analysis)

- Absorbances > 1.1 x $OD_{Cut\text{-}Off}$ have to be considered as positive.
- Absorbances < 0.9 x $OD_{Cut\text{-}Off}$ have to be considered as negative.
- Absorbances ≥ 0.9 x $OD_{Cut\text{-}Off}$ and ≤ 1.1 x $OD_{Cut\text{-}Off}$ have to be considered as equivocal.

Calculation Example

Row	Antigen	OD_{CAL}	Factor	$OD_{Cut\text{-}Off}$	OD_{Sample}	Result
A	SS-A/Ro	1.393	0.21	0.293	1.379	Pos
B	SS-B/La	0.562	0.55	0.309	2.442	Pos
C	SmD1	0.843	0.58	0.489	0.056	Neg
D	U1-snRNP	1.069	0.33	0.353	0.060	Neg
E	Histone	1.009	0.38	0.383	0.100	Neg
F	CENP-B	1.089	0.30	0.327	0.044	Neg
G	Scl70	1.025	0.44	0.451	0.096	Neg
H	Jo1	0.870	0.43	0.374	0.055	Neg

Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

Elevated antinuclear antibodies may occur in individuals with no evidence of clinical disease.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

The performance characteristics for this assay have not been established for plasma samples.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/el-60033.pdf or

www.human-de.com/data/gb/vr/el-60033.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

STOP **Warning**

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

SUB **Danger**

· Hazard statements

H360D May damage the unborn child.

· Precautionary statements

CAL NC PC WASH 20x DIL CON SUB STOP

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Damoiseaux J.G., Tervaert J.W.., Autoimmun Rev. **5**, 10-17 (2006)
2. Egner W., J. Clin. Pathol. **53**, 424-432 (2000)
3. Conrad K. *et al.*, Autoantibodies in Systemic Autoimmune Diseases – A Diagnostic Reference; Pabst Science Publishers, Lengerich, Berlin, Riga, Rom, Viernheim, Wien, Zagreb, 2002

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