DRG® ENA Combi (EIA-3572)

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Revised 29 Dec. 2009 rm (Vers. 3.0) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

ENA Combi is an indirect solid phase enzyme immunoassay (ELISA) for the semi-quantitative measurement of IgG class autoantibodies against extractable nuclear antigens (ENA) in human serum or plasma.
The assay is intended for in vitro use only as an aid in the determination of rheumatic diseases such as systemic lupus erythematosus (SLE), Sjögren´s syndrome, scleroderma and mixed connective tissue disease.

SUMMARY AND EXPLANATION OF THE TEST

Rheumatoid autoimmune diseases are often associated with the occurrence of autoantibodies against several nuclear or cytoplasmatic antigens. These so-called anti nuclear antibodies (ANA) can be divided into three groups:

1. true anti nuclear antibodies (ANA):
   directed against dsDNA, ssDNA, histones, nucleolic RNA and DNP
2. extractable nuclear antibodies:
   directed against Sm (Smith), n-RNP, Scl 70, Jo-1 and PM-1
3. cytoplasmatic antibodies:
   directed against SS-A (Ro) and SS-B (La)

In patients with Sjögren-Syndrome antibodies against the two cytoplasmatic antigens often occur in combination. Due to their strong association of SS-A and SS-B antibodies to the HLA-DR3 and DR2 phenotypes a genetic predisposition is suspected. The anti SS-A protein passes the placenta and may cause the development of SLE in neonates.
Immunoreactive proteins may occur in various combinations and also bound to 'host proteins' of viral origin. They induce synthesis of polyclonal autoantibodies of the IgG, IgM and IgA class of immunoglobulins. Especially for mixed connective tissue diseases a relation to viral infec-tions by EBV (Eppstein-Barr-Virus) is indicated.
Each class of immunoglobulins causes specific immune fluorescent pattern. Basically immunofluorescence titers correlate with the quantitation of IgG antibodies but the concentrations may considerably vary within each titer. Quantitation of IgG class antibodies extensively cor-relates with the diseases' activity. This makes it superior to immunofluorescence using HEp-2 cells, which may give variable results depending on their degree of activity. Also IF with Crithidia luciliae sometimes gives discrepant results.
Today the best investigated immunoreactive antigens are double-stranded DNA (dsDNA), single stranded DNA (ssDNA), Sm (Smith), sn-RNP (small nuclear ribonucleoprotein particles), the complex RNP/Sm which is stabilized by ribonucleic acid as well as SS-A (Ro) and SS-B (La). The antigen Scl 70, a 70 kD molecular weight protein is associated with scleroderma.
In rheumatoid autoimmune diseases various profiles of autoantibodies to these antigens can be detected. In a high incidence they are related to active and inactive systemic Lupus erythrematosus, mixed connective tissue diseases (Sharp Syndrome), rheumatoid arthritis, Sjögren-Syndrome, Sclerodermia, photosensitive dermatitis and drug-induced lupus.
In Lupus patients typically anti-dsDNA antibodies can be detected. Patients without these antibodies very often show anti-ssDNA antibodies and anti-SS-A and anti-SS-B are present. A strong correlation between antibody concentration and severity of the disease has been observed with higher antibody concentrations in active phases of the disease. Thus quantitation is more informative compared to simple titering by immunofluorescence.

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Most of these parameters are not specific for just one disease but they occur in various combinations. The pattern of different antibody combinations and their concentration together with the whole clinical picture of the patient are helpful diagnostic tools in the assessment of rheumatoid autoimmune diseases.
The following graph gives brief information on the complexity of autoimmune disease occur-ring antibodies. It is not designed as a diagnostic schedule or program for ongoing diagnostic profiles:

Diseases	Anti								
	dsDNA	ssDNA	Histone	SS-A (Ro)	SS-B (La)	Sm	RNP/Sm	Scl 70	Jo-1
Systemic Lupus erythrmatodes (SLE)	xxx	xx		x	x	xx	x		
Drug induced Lupus (LE)		xx	xxx						
Sharp-Syndrome/Mixed connective tissue diseases	x	x		x	x	xx	xxx		
Rheumatoid Arthritis	x	xx	x						
Sjören's - Syndrome	x	x		xx	xx				
Scleroderma	x		x			x		xx	x
Photosens. Dermatitis Dermatomyositis	xx			x					xx

PRINCIPLE OF THE TEST

Extractable nuclear antigens (SS-A (Ro), SS-B (La), Sm, RNP/Sm, Scl 70 and Jo-1) are bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG anti-bodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro determinations only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.

5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size 96 determinations

Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified extractable nuclear antigens (ENA): reference antigen (row A and B), SS-A (row C), SS-B (row D), Sm (row E), RNP/Sm (row F), Scl 70 (row G) and Jo-1 (row H). Ready to use.
4 vials, 1.5 ml each	Anti-ENA Controls in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1%(w/w)) Negative Control (A) (12,5 U/ml), Cut-Off Control (B) (25 U/ml), Positive Control (C) (50 U/ml), High positive Control (D) (100 U/ml), approximate units are printed on the labels. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (contains acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN_3 <0.1% (w/w)), concentrate (50x).

Revised 29 Dec. 2009 rm (Vers. 3.0) **RUO in the USA**

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
- Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
- Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).

4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

Revised 29 Dec. 2009 rm (Vers. 3.0) **RUO in the USA**

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of controls and prediluted patient samples in duplicate into the wells.

		1	2	3	4	5	6	
mixed antigen	**a**	CA	CC					CA - CD Controls A to D
mixed antigen	**b**	CB	CD					
SS-A	**c**	P1	P2	P3	P..			P1, P2,.. Patient sample
SS-B	**d**	P1	P2	P3	P..			
Sm	**e**	P1	P2	P3				
RNP/Sm	**f**	P1	P2	P3				
Scl 70	**g**	P1	P2	P3				
Jo-1	**h**	P1	P2	P3				

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash **3 times** with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash **3 times** with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The ENA Combi ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Negative Control (A), Cut-Off Control (B), Positive Control (C) and High Positive Control (D) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Qualitative evaluation of ELISA

Evaluation of the ENA Combi test is carried out by direct comparison of the optical density of each patient sample with the optical density of the Cut-Off Control.
Patient samples exhibiting optical densities higher than the optical density of the Cut-Off Control are considered to be positive.

Negative:	OD Patient	<	OD Cut-Off Control
Positive:	OD Patient	>	OD Cut-Off Control
Strong Positive:	OD Patient	>>	OD Strong Positive Control

Quantitative evaluation of ELISA

For quantitative calculation of the patients results the concentration of the controls may be used for creating a calibration curve. The concentration of unknowns may be estimated from this calibration curve.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the ENA Combi test kit:

	ENA Combi (U/ml)
negative:	< 25
positive:	>25

Further differentiation and typing should be carried out using fully quantitative Anti-ENA test kits.
Positive results should be verified concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of ENA antibodies in serum. The above reference ranges should be regarded as guidelines only.

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PERFORMANCE CHARACTERISTICS

Specificity

The microplate is coated with the antigens SS-A (Ro), SS-B (La), Sm, RNP/Sm, Scl 70, Jo-1. All antigen preparations are highly purified by affinity chromatography. The ENA Combi test is specific only for autoantibodies directed to these antigens. No cross reactivities have been observed.

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA, since no other international standards are available.

LIMITATIONS OF PROCEDURE

Not all patients with SLE, Sjögren´s syndrome or connective tissue disease are positive for anti-bodies against ENAs The ENA Combi ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

REFERENCES/Literature

1. Antinuclear Antibody The Lancet, September 15, 1984: 611 - 13.
2. Froelich, Ch. J., Wallmann, H., Skosey, J. L. and Teodorescu, M. Clinical Value of an Integrated ELISA System for the Detection of 6 Autoantibodies. The Journal of Rheumatology 1990; 17:2: 192 - 200.
3. Harley, J. B. and Gaither, K. K. Autoantibody. Rheumatic Disease Clinics of North America April 1988; Vol. 14, No. 1: 43 - 55.
4. Hartung, K. and Deicher, H. Systemischer Lupus erythematodes. Allergologie Nr. 7/1985; Jahrgang 8: 275 - 281.
5. Hartung, K. and Deicher, H. Symptome und Symptommuster beim systemischen Lupus erythematodes. Die Internistische Welt 1987; 9: 236 - 40.
6. Kühl, R., Sachs,V., Dörner, R., Utecht,B. und Gross,W. Ein Enzymimmunoassay als Suchtest für Antikörper gegen extrahierbare Kernantigene. Äerztl. Lab. 1987; 33: 5 - 9.
7. Reichlin, M. and Van Venrooij,W. J. Autoantibodies to the URNP particles: relationship to clinical diagnosis and nephritis. Clin. exp. Immunol. 1991; 83: 286 - 90.

8. Tzioufas, A. G., Manoussakis, M. N., Drosos, A. A., Silis, G., Gharavi, A. E. and Moutsopoulos, H. M. Enzyme immunoassay for the detection of IgG and IgM anti-dsDNA antibodies: clinical significance and specificity. Clinical and Experimental Rheumatology 1987; 5: 247 - 253.

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Symbols used in DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..