

EIAgen ENA- Screen KIT

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96 Tests

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




FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2...8 °C



EN

SYMBOLS USED ON LABELS

[IVD]	In vitro diagnostic medical device (In vitro diagnostic use)
[LOT]	Lot number
[REF]	Catalogue Code
[MT_PLATE]	Microplate
[CONJ HRP]	Conjugate
[CAL_1]	Calibrator 1
[CAL_2]	Calibrator 2
[CAL_3]	Calibrator 3
[CAL_4]	Calibrator 4
[CONTROL +]	Positive Control
[CONTROL -]	Negative Control
[SOLN TMB]	Substrate (TMB)
[DILSPE]	Sample Diluent
[BUF WASH 20x]	Washing Buffer 20X
[SOLN STOP]	Stop Solution
	Expiry date (Use by...)
 2°C 8°C	Temperature limitation (store at 2...8°C)
	Number of tests
	Keep away from sunlight
M	Manufactured by
	Attention, See Instructions For Use
	Biological risk

1.0 INTENDED USE

The EIAgen ENA Screen is an enzyme-linked immunosorbent assay method for the semi-quantitative determination of specific IgG autoantibodies to SS-A/Ro, SS-B/La, Sm, Sm/RNP, Jo-1 and Scl-70 in human serum. The results of the anti-ENA 6 Screen assays can be used as an aid in the diagnosis of auto-immune diseases including Systemic Lupus Erythematosus (SLE), Sjogren's Syndrome (SS), Mixed Connective Tissue Disease (MCTD), Poly/Dermato myositis (PM/DM) and Scleroderma. Levels of these autoantibodies are one indicator in a multi-factorial diagnostic regime.

2.0 SUMMARY AND EXPLANATION OF THE TEST

The detection of Anti-Nuclear Antibodies (ANA's) has long been an important tool in the diagnosis of systemic rheumatic diseases. The antigens used in their detection are purified by the saline extraction of human or animal nuclei, this has led to them being termed Extractable Nuclear Antigens (ENA's). The most commonly measured ENA specifications are anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-Sm/RNP, anti-Jo-1 and anti-Scl-70.

The intracellular antigens SS-A/Ro, SS-B/La, Sm, Sm/RNP, Jo-1 and Scl-70 are targets for autoimmune responses in many patients with rheumatic diseases. Anti-SS-A/Ro antibodies are found in 30-50% of patients with Systemic Lupus Erythematosus (SLE), but most significantly in around 95% of patients with primary or secondary Sjogren's Syndrome (SS). Anti-SS-B/La antibodies are also found in SLE and SS patients. Anti-Sm antibodies are considered highly specific for SLE and approximately 30-40% of patients show their presence. Anti-RNP antibodies are predominantly found in patients with Mixed Connective Tissue Disease (MCTD) but are also associated with SLE, SS and Scleroderma. Anti-Jo-1 antibodies are considered specific for Polymyositis and Dermatomyositis. Anti-Scl-70 antibodies are recognised as specific markers for Primary Systemic Sclerosis (PSS or Scleroderma).

Autoantibodies to ENA's vary depending on disease state. The following are estimated incidences of ENA antibodies in various diseases:

Antibody	SLE	SS	PSS	MCTD	PM	DM
SS-A/Ro	30-50%	~95%	---	---	---	---
SS-B/La	>15%	~87%	---	---	---	---
Sm	30-40%	---	---	---	---	---
Sm/RNP	35-45%	>30%	>20%	95-100%	---	---
Jo-1	-----	---	---	>25%	>25%	---
Scl-70	-----	20-30%	---	---	---	---

3.0 PRINCIPLE OF THE ASSAY

The EIAgen ENA Screen assay for detection of autoantibodies is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a colour reaction of an enzyme and substrate. The EIAgen wells are coated with purified antigens. On adding diluted serum to the wells the antibodies present bind to the antigen. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG monoclonal antibody is added, which binds to the immobilised antibodies.

Following further incubation and washing, tetra-methyl benzidine substrate (TMB) is added to each well. The presence of the antigen-antibody-conjugate complex turns the substrate to a dark blue colour. Addition of the stop solution turns the colour to yellow.

The colour intensity is proportional to the amount of autoantibodies present in the original serum sample.

4.0 KIT COMPONENTS

4.1 MICROPLATE (code 52PIA) [MT_PLATE]

1 microplate is supplied which contains 12 strips of 8 breakapart wells. The wells are coated with ENA purified from bovine sources. All six antigens are present in each well.

4.2 CONJUGATE (code 52HRP) [CONJ|HRP]

1 vial containing 15ml of ready-to-use HRP conjugate. Conjugate contains 0.05% Proclin 300. Conjugates are color coded pink.

4.3 CALIBRATORS

4 vials containing 1ml of calibrator. The calibrators are calibrated to arbitrary units and contain human antisera. The calibrators contain 0.09% sodium azide as a preservative. The concentrations allocated to the calibrators are:

Calibrator	symbol	code	concentration
Calibrator 1	[CAL_1]	52CAL1	5 u/mL
Calibrator 2	[CAL_2]	52CAL2	20 u/mL
Calibrator 3	[CAL_3]	52CAL3	50 u/mL
Calibrator 4	[CAL_4]	52CAL4	100 u/mL

4.4 POSITIVE CONTROL (code 52POS) [CONTROL|+]

1 vial containing 0.45 ml of concentrated positive control which contains human antisera and 0.09% sodium azide as a preservative.

4.5 NEGATIVE CONTROL (code 52NEG) [CONTROL|-]

1 vial containing 0.45 ml of concentrated negative control which contains normal human serum and 0.09% sodium azide as a preservative.

The controls (positive and negative) are provided in a concentrated form and should be diluted 1/100 with sample diluent buffer before use. Prepare fresh control dilutions before each assay run. Vortex all samples and controls before testing.

4.6 SUBSTRATE (TMB) (code TMBC) [SOLN|TMB]

1 vial containing 15ml of ready-to-use tetra-methylbenzidine (TMB) substrate.

4.7 SAMPLE DILUENT (code 52SD) [DILSPE]

2 bottles containing 60ml/each of ready-to-use sample diluent buffer. The buffer includes 0.09% Sodium azide. Sample diluent buffer is color coded blue.

4.8 WASHING BUFFER 20X (code TLAVC) [BUF|WASH|20x]

1 bottle containing 50ml of wash buffer concentrate. Wash buffer concentrate contains 0.06% Proclin 300.

Dilute the whole content of the bottle up to one liter with distilled or deionized water. Mix well before use. Store this solution at 2-8°C if it is not to be used immediately. The diluted wash buffer is stable at 2-8°C for one week.

4.9 STOP SOLUTION (code STOPC) [SOLN|STOP]

1 bottle containing 20ml of 0.25M H₂SO₄ stop solution.

The MSDS is available upon request of laboratory personnel.

5.0 STORAGE AND STABILITY AFTER THE FIRST OPENING

- Store kit components at 2-8°C and do not use after the expiry date on the box outer label.
- Before use all components should be allowed to warm up to ambient temperature (18...25°C).
- After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C.
- The opened kit should be used within three months.

6.0 MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- Wash bottle, automated or semi-automated microwell plate washing system.
- Rack for sample dilution.
- Micropipettes including multichannels capable of accurately delivering 5-1000µl (less than 3% cv).
- Reagent reservoirs for multichannel pipettes.
- One-liter graduated cylinder.
- Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950ml water).
- Microtiter plate reader equipped for the measurement of the absorbance at 450 and 405 nm (reference filter at 620 nm).
- Paper towels, pipette tips and timer

7.0 WARNINGS OR PRECAUTIONS

7.1 SAFETY PRECAUTIONS

- All reagents in this kit are for in vitro diagnostic use only
- Only experienced laboratory personnel should use this test and handling should be agreement with GLP.
- Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- Reagents contain preservatives which may be toxic if ingested. Do not pipette by mouth. Avoid contact of reagents or patient samples with skin or mucous membranes. If contact occurs, immediately flush with large quantities of water. Avoid splashing or creation of aerosols. Reusable glassware must be thoroughly washed and rinsed so that it is free of all detergents.
- Sera used in the preparation of the calibrators and controls have been tested for the presence of antibodies to Human Immunodeficiency Virus (HIV 1 and 2), as well as for Hepatitis B Surface Antigen (HBsAg) and HCV and found to be negative. All material is tested with FDA approved assays. Because no test method can offer complete assurance that HIV, HBsAg or other infectious agents are absent, it is recommended that human serum based products be handled with the same precautions used for patient specimens.
- Dispose of reagent solutions containing sodium azide and thimerosal as preservatives according to all local, state and national regulations. To dispose of reagents containing azide, flush away using copious amounts of water. Dispose with caution as sodium azide can form explosive compounds on prolonged contact with lead or copper piping.

7.2 TECHNICAL PRECAUTIONS

A. Correct use of reagents and proper pipetting

- The performance data represented here were obtained using specific reagents listed in the package insert. Do not use reagents from other manufacturers in the kits.
- Do not use reagents from other EIAgen kits with this kit. Do not mix reagents from different kit lots.
- Do not dilute or adulterate the kit reagents, unless directed by the kit protocol.
- Do not use the substrate solution if it has begun to turn blue.
- Do not use heat-inactivated serum.
- Microplate washing is important. Improperly washed wells will give erroneous results

B. Adherence to assay procedure and specifications

The obtained values have to be always compared to the ones reported in QC sheet. Do not use the kit to determine values outside the range indicated in the IFU.

The test protocol must be followed strictly. Observe the indicated incubation times and temperature and the washing procedure. These are critical steps.

Include the positive and negative controls in every test run to monitor for reagent stability and correct assay performance. Please refer also to section 10.2_Quality Control.

8.0 SPECIMEN COLLECTION AND STORAGE

- Use serum in this procedure.
- It is most important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Obtain patient samples by non-traumatic venipuncture, using a vacuum tube or sterile syringe. If a syringe is used, transfer the blood immediately to a vacuum tube (plain red-top or serum separator).
- Allow samples to clot at room temperature (18...25°C) for at least 20-30 minutes, until the clot just begins to retract. Spin the sample in a centrifuge. Immediately following the centrifugation, transfer the cell-free serum to a tightly stoppered storage bottle.
- Do not use sera samples showing signs of haemolysis. If it is necessary to store a sample prior to analysis, it is recommended that, for a period of up to 72 hours, store the sample in a sealed container at 2-8°C. Freeze samples at -20°C if longer storage is required.
- Avoid repeat freeze-thawing.

9.0 ASSAY PROCEDURE

9.1 REAGENT PREPARATION

Bring all reagents to room temperature (18...25°C).

Select sufficient microwells for the test. Remove protective covering and select sufficient wells to accommodate the patient samples, calibrators and assay controls. Each sample is recommended to be tested in duplicate.

Dilute all serum samples and assay controls 1/100 in sample diluent by adding 10µl to 990µl sample diluent. Calibrators do not require dilution.

9.2 PIPETTING AND INCUBATION STEPS

- A. Pipette 100µl of the calibrators, diluted control or diluted patient sample into the wells. Dispense 100µl of sample diluent as zero calibrator in the first well.
- B. Incubate the wells at room temperature (18...25°C) for 30 minutes.
- C. Wash the wells three times as described in section 9.3_PROCEDURAL NOTES.
- D. Add 100µl of ready-to-use conjugate to each well.
- E. Incubate the wells at room temperature (18...25°C) for 15 minutes.
- F. Repeat washing as in section C above.
- G. Add 100µl of ready-to-use TMB substrate to each well.
- H. Incubate the wells at room temperature (18...25°C) for 15 minutes.
- I. Add 50µl of stop solution to each well. Tap gently to ensure uniform color distribution and read within 15 minutes.
- J. To read the plate, ensure the base is free from moisture and no air bubbles are in the wells. Read the absorbance of the well contents at 450nm on a suitable plate reader. On readers equipped with a dual wavelength facility set the reference filter to 600 – 650 nm.
- K. Subtract the blank from the optical densities of the calibrator, controls and patient samples. If the assay was performed in duplicate, the mean of the wells should be taken.

9.3 PROCEDURAL NOTES

Do not allow the wells to dry between incubations.

Do not vary reagents and incubation temperatures above or below room temperature (18...25°C).

WASHING PROCEDURE

The washing procedure can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel.

10.0 CALCULATION OF RESULTS

10.1 VALIDITY OF THE ASSAY

In order that the assay be valid, the following criteria must be fulfilled:

- Adaltis supplies with each EIAgen ENA Screen kit, positive and negative control samples which should be assayed with each run. The results of these quality control samples should fall within the limits indicated on the Certificate of Analysis.
- Results obtained for quality control sera must fall within acceptable ranges: please refer also to next section 10.2_QUALITY CONTROL.

10.2 QUALITY CONTROL

Good laboratory practice indicates that with each assay run, one or more quality control samples of known antibody level should be analyzed as though they were clinical samples. Positive and negative control sample are supplied with each kit which may be assayed with each run. The results of these quality control samples should fall within the limits indicated on the Certificate of Analysis.

Should the results fall outside this range repeat the assay using freshly prepared controls. Should the results continue to fall outside the specific range, and after equipment, adherence to the protocol and laboratory procedure have been verified, seek assistance from the supplier. Do not report patient results if the control results fall outside the acceptable ranges.

10.3 OD CONVERSION

Optical densities (ODs) higher than 2.0 are out of the measurement range of some microplate readers. It is therefore necessary, for ODs higher than 2.0, to perform a reading at 405nm (= wavelength of peak shoulder) in addition to 450nm (peak wavelength) in addition to 450nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm
- Read again the plate at 405 nm and 620 nm
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/Od 405 = 3.0), that is:

$$OD\ 450\ nm = OD\ 405\ nm \times 3.0$$

Warning: the conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his own reader.

10.4 QUALITATIVE CALCULATION

Calculate the absorbance value (OD), blank corrected, for the kit calibrator and controls. Calculate the mean, blank corrected, absorbance value (OD) for duplicates of the patient samples. Using the following algorithm, calculate the concentration of each of the samples:

$$\frac{\text{Concentration of calibrator 2}}{\text{OD of calibrator 2}} \times \text{OD of sample or control}$$

The concentration of calibrator 2 is 20 u/ml.

This method provides a qualitative result only

10.5 SEMI-QUANTITATIVE CALCULATION

Data Reduction – manual method

Plot the optical densities (OD's) of the calibrators against the concentration values, using a linear y-axis (OD) and a logarithmic x-axis (concentration). Use the reagent blank as the zero calibrator. The concentration value of the patient samples can then be determined from this calibration curve.

Data reduction – automated method

Alternatively, use a 4-parameter logistic curve fitting for the calibrator curve and for calculating results, using a log scale for the x-axis and a linear scale for the y-axis.

11.0 EXPECTED VALUES

A total of 217 samples were assayed. 50 of the samples were from normal subjects and 167 from people with an autoimmune condition. The cut-offs shown below were based on 94 samples tested.

U/ml	Negative	Equivocal	Positive
ENA-6 Screen	<8	8 - 10	>10

It is recommended that equivocal samples be retested using a subsequent sample.

It is recommended that positive samples be tested for specific ENA antibodies.

12.0 LIMITATIONS OF PROCEDURE

12.1 KNOWN INTERFERENCES

Grossly haemolysed, lipaemic or microbiologically contaminated samples should not be used. Samples with abnormally elevated levels of haemoglobin, bilirubin and especially EDTA may interfere with assay performance and accuracy.

A 'hook effect' may only be seen with very high samples which are above the assay range. No hook effect is seen up to 2355.2 U/ml. This is a sample specific effect.

12.2 CAUTIONS IN INTERPRETATIONS OF THE RESULTS

A negative result should not be used as a sole criterion to rule out connective tissue disease or other autoimmune disease, but must be taken in relation to other clinical observations and diagnostic tests. While the precision of the ElAgen kit is sufficient to allow samples to be measured in single determinations, this is done at the clinical laboratory's discretion. It is advised that duplicate determinations should be used to enable identification of potential pipetting error or to allow for confirmation in the equivocal range.

It is recommended that positive samples be tested for specific ENA antibodies. It should be noted that ENA antibodies occur at low levels in other autoimmune and non-autoimmune conditions. Therefore all other clinical observations and diagnostic tests should be taken into account for clinical diagnosis.

13.0 PERFORMANCE CHARACTERISTICS

13.1 PRECISION

Intra- and inter- assay variation were checked using a number of samples.

Intra-assay variation:	A	B	C
x	15.3	31.9	68.4
%cv	4.0	3.7	6.4

Inter-assay variation:	A	B	C
x	15.3	31.9	68.4
%cv	8.9	8.5	12.9

13.2 ANALYTICAL SPECIFICITY AND SENSITIVITY

Of a panel of 29 'normal' asymptomatic individuals 100% gave a negative result. No cross-reactivity with anti-GBM, anti-thyroglobulin, anti-TPO, anti-gastric parietal cell, anti-cardiolipin or ANCA was seen.

The sensitivity of the assay was established by calculation of the mean plus two standard deviations of a minimum of 20 replicates of the zero calibrator which gave a value of 0.57 U/ml.

13.3 RELATIVE SPECIFICITY AND SENSITIVITY

The manual assay was compared to another commercially available test and was found to be substantially equivalent. The results are shown below:

Predicate	Manual	
	Positive	Negative
Positive	30	5
Negative	0	20
Relative sensitivity	= 85.7 %	
Relative specificity	= 100 %	
Overall agreement	= 90.9 %	

Equivocal results were omitted from the above calculations.

14.0 AUTOMATION

Application protocols for the proper automation on the Adaltis microplate analyzers are available upon request at Adaltis directly.

15.0 SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors .

ERROR	POSSIBLE CAUSES / SUGGESTIONS
OD very different (± 50%) from OD reported on QC	<ul style="list-style-type: none"> - incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) - incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance; note down the beginning of the incubation) - error in washing or in spectrophotometer reading (suggestion: check operating or settings of respective instruments) - contamination of Substrate (suggestion: use only disposable and clean plastic containers)
Low reproducible results	<ul style="list-style-type: none"> - incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) - incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance) - error in washing or in reading to spectrophotometer (suggestion: check operating or settings of respective instruments) - contamination of Substrate (suggestion: use only disposable and clean plastic containers) - pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water)
no colourimetric reaction after addition of substrate	<ul style="list-style-type: none"> - no reagent pipetted - strong contamination of conjugate or Substrate - errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)
too low reaction (too low ODs)	<ul style="list-style-type: none"> - incorrect conjugate (e.g. not from original kit) - incubation time too short, incubation temperature too low
too high reaction (too high ODs)	<ul style="list-style-type: none"> - incorrect conjugate (e.g. not from original kit) - accidental contamination/degradation of conjugate - incubation time too long, incubation temperature too high - water quality for wash buffer insufficient (low grade of deionization) - insufficient washing (conjugates not properly removed)
unexplainable outliers	<ul style="list-style-type: none"> - contamination of pipettes, tips or containers - insufficient washing (conjugates not properly removed)
too high within-run CV%	<ul style="list-style-type: none"> - reagents and/or strips not pre-warmed to Room Temp. prior to use - plate washer is not washing correctly (suggestion: clean washer head)
too high between-run CV%	<ul style="list-style-type: none"> - incubation conditions not constant (time, temperature) - controls and samples not dispensed at the same time (with the same intervals) - (check pipetting order) - person-related variation

16.0 BIBLIOGRAPHY

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