PICTORLIMITED
ACCESSIBLE DIAGNOSTIC TECHNOLOGY
FOR A HEALTHY WORLD

PICTARRAY™
ENA
Instructions

PictArray™ Extractable Nuclear Antigen (ENA) ELISA Kit

Instructions for testing human serum samples on PictArray™ ENA Panel
consisting of RNP/Sm, SSA (Ro60), SSA (Ro52),
SSB, Jo1, Sm, Scl70, CENP-B and dsDNA.

PLEASE READ COMPLETE INSTRUCTIONS BEFORE USING THIS KIT

1. Intended Use

PictArray ENA Panel is a miniaturized immunoassay for detection of IgG antibodies in human sera to nine autoantigens. This in vitro test is intended as an aid in the diagnosis of autoimmune connective tissue diseases. PictArray results can be reported either for individual tests or for the complete panel.

Layout for PictArray ENA Panel

	RNP/ Sm	Systemic Lupus Erythematosus& Mixed Connective Tissue Disease
	SSA (Ro60)	Systemic Lupus Erythematosus & Sjogren's Syndrome
	SSA (Ro52)	Systemic Lupus Erythematosus & Sjogren's Syndrome
	SSB	Systemic Lupus Erythematosus & Sjogren's Syndrome
	dsDNA	Systemic Lupus Erythematosus
	Jo1	Myositis
	Sm	Systemic Lupus Erythematosus
	Scl70	Systemic Sclerosis (Scleroderma), diffuse
	CENP-B	CREST syndrome, Systemic Sclerosis (Scleroderma), limited

2. Clinical Significance

Patients with autoimmune connective tissue disorder present with a set of common clinical symptoms. Identification of individual autoantibody signatures is an invaluable aid to diagnose the specific type of disorder. The following table summarizes the various autoantibodies with respect to disease association:

Antigen reactivity	Disease state	Frequency in patients with disease	Reference
RNP/ Sm	Mixed connective tissue disease; Systemic lupus erythematosus (SLE)	40% (SLE)	Mongey AB & Hess EV (1989) Adv. Int. Med. 36, 151.
SSA (Ro60)	SLE; Sjögren's Syndrome (SS)	30 to 40% (SLE) 50 to 80% (SS)	Harly JB et al (1986) Arth. Rheum. 29, 196
SSA (Ro52)	SLE; Sjögren's Syndrome	30 to 40% (SLE) 50 to 80% (SS)	Harly JB et al (1986) Arth. Rheum. 29, 196
SSB	SLE; Sjögren's Syndrome	87%	Harly JB et al (1986) Arth. Rheum. 29, 196
dsDNA	SLE	60 to 80%	Hochberg MC (1997) Arth Rheum. 40, 1725
Jo1	Myositis	15 to 20%	Nishikai M. Reichlin M (1980) Arth. Rheum. 23, 881
Sm	SLE	25%	Notman DD et al (1975) Ann. Intern. Med 83, 464
Scl70	Diffuse scleroderma	20 to 28%	Douvas AS et al (1979) J. Biol. Chem. 254, 10514.
CENP-B	Limited form of scleroderma	48%	Russo et al (2000) J. Rheumatol, 27, 142.

PictArrays provide an efficient method for screening patients with autoimmune connective tissue disease by simultaneously testing reactivity to nine antigens. The analysis of test data by computer-generated algorithms reduces subjectivity in interpretation of results. Internal controls are included in every test to monitor performance reducing the chances of human and mechanical errors as compared to a conventional ELISA assay done using one well-one test microtiter plates.

3. Test principle

The detection of antibodies to pathogenic antigens is based on the ELISA principle.

a. Diluted serum samples are incubated in wells containing duplicate spots of nine different nuclear antigens and seven test controls.
b. After washing non-bound serum components, binding of IgG is detected by sequential incubations with biotin-labelled anti-human IgG, followed by a Streptavidin-peroxidase conjugate.
c. The free conjugates are washed off and enzymatic activity bound to the antigen spots is measured by incubation with substrate.
d. The reaction is stopped by washing off excess substrate and the slide is imaged. The color intensity of each spot is directly proportional to the amount of antibody bound to the antigen.

1 SIMPLE 2 AFFORDABLE 3 ACCURATE

4. Kit components

Component	Tube Label	Description		
PictArray slides		Test slide with 8 two-well modules containing 300 µ spots of test reagents	1 slide	3 slides
Wash Buffer 10X	WASH	Buffer containing phosphate buffered saline with Tween 20	3.1mL	12.0mL
Diluent 10X	DIL	Buffer containing blocking agent and phosphate buffered saline with Tween 20	1.1mL	7.0mL
Conjugate G 20X	CONJ G	Anti-human IgG antibody conjugated to biotin	0.15mL	0.3mL
Detection reagent 20X	DET	Streptavidin conjugated to horseradish peroxidase	0.15mL	0.3mL
Substrate A 20X	SUBS A	Solution containing diaminobenzidine tetrahydrochloride	0.15mL	0.3mL
Substrate B 1X	SUBS B	Buffer containing Hydrogen peroxide	2.0mL	6.0mL
Pack insert		Protocol for using the PictArray ENA ELISA test kit	1	1

5. Storage conditions

Store the unopened kit immediately upon arrival at 4 °C. DO NOT FREEZE.

6. Materials required but not provided

6.1 For laboratories manually processing moderate numbers of samples

Deionized or distilled water

Precision pipettes to deliver from 5 µl to 200 µl volumes.

Adjustable 1 ml pipettes for reagent and sample preparation.

Incubator set at 37 °C.

PictImager for reading test results

Computer installed with Pictorial Image Analysis software.

6.2 For laboratories with automated sample processing flow

NOTE:

A specially-designed frame to hold up to four 16-well slides is available from Pictor. This reusable frame fits into most commercially available ELISA plate processors

Deionized or distilled water

Automated ELISA plate washers or analyzers programmed to process PictArray slides.

PictImager for reading test results

Computer installed with Pictorial Image Analysis software.

7. Precautions

7.1 Safety precautions

All reagents in this kit are for *in vitro* diagnostic use only.

Handle all human material as if potentially infectious. Users should wear gloves and protective clothing when handling any patient sera or serum-based products.

Avoid any contact of the reagents in the kit with the skin and mucosa (toxicity, irritation and burn hazard). Material safety data sheets for kit components are available upon request.

Handle and dispose samples and reagents in accordance with local legislation and established laboratory protocols.

7.2 Technical precautions

Follow complete instructions for proper performance of the kit and to obtain reliable results.

Ensure that the slide and/or the two-well modules are correctly aligned with the sample layout before adding samples.

Only freshly drawn and properly refrigerated sera should be used in this assay. Sera may be stored between 2 °C and 8 °C for no longer than 48 hours. If delay in testing is anticipated, the sera should be stored at -20 °C.

1 SIMPLE 2 AFFORDABLE 3 ACCURATE

Do not allow the wells in the slide to dry between any of the steps during sample processing until the final wash step is performed.

It is important to add samples and reagents into wells without any delay. Please ensure that all the samples and reagents are ready to dispense.

Use a different pipette tip for each sample and change tips for addition of different reagents. Cross contamination of reagents and/ or samples can lead to incorrect results.

8. Preparation of Reagents

NOTE:

The volumes given in the table below are sufficient to process one slide. Volumes should be increased proportionally to test more slides.

Keep undiluted stock reagents at 4°C immediately after use.

Diluted 1X Wash Buffer and 1X Diluent are stable for 3 months when stored at 4°C.

All other diluted 1X reagents must be used within 2 hours.

1. **Spin all tubes in the kit briefly before use.**

2. **1X Wash Buffer (WASH)**

NOTE:

If crystals are visible, warm the tube at 37 °C for 10 minutes.

Add 4ml of 10X Wash Buffer into a 50ml cylinder.

Add distilled water to make volume to 40ml.

Mix well and transfer into a bottle labelled “1x Wash Buffer”.

Store at 4 °C.

3. **1X Diluent (DIL)**

Add 1.0ml of 10X Diluent into a 10ml cylinder.

Add 9.0ml of **1X Wash Buffer** to make volume to 10ml.

Mix well and transfer into a labelled “1X Diluent” 10ml polypropylene tube.

Store at 4 °C.

4. **0.1X Sample Diluent (DIL)**

Add 0.5ml of **1X Diluent** into a 5ml cylinder.

Add 4.5ml of **1X Wash Buffer** to make volume to 5ml.

Mix well and transfer into a 10ml polypropylene tube labelled “0.1X Sample Diluent”.

Store at 4 °C.

9. Test Protocol

NOTES:

1. 2-well modules may be processed individually. Unused modules should be kept refrigerated.

2. Please refer to “Custom Sample Layout” section in the Pictorial Image Analysis Manual

1. **Sample Layout**

Please follow instructions in the Pictorial© Image Analysis Instruction Manual to generate sample layout.

2. **Preparation of human serum samples**

Label one set of tubes depending on the number of samples to be tested.

Add 45µl of 0.1X Sample Diluent to each tube.

Add 5µl of serum sample to each tube starting from Tube 1.

Vortex gently and spin briefly.

This gives a 10-fold dilution of each sample.

Label a second set of tubes.

Add 90µl of 0.1X Sample Diluent to each tube.

Add 10µl of 10-fold diluted serum sample to each tube starting from Tube 1.

Vortex gently and spin briefly.

This gives a 100-fold dilution of each sample.

3. Addition of human serum samples to PictArrays

Remove the required number of modules and attach to the slide backing.

Touch only the corner of the wells with the pipette tips to avoid damaging the well surface.

Ensure that the notch on the upper left side of the slide aligns with the layout.

Add 50µl of the appropriately diluted sample to array wells according to the printed layout.

NOTE:

Add 0.1X Sample Diluent to the well not containing sample, if single sample is to be tested.

Place the slide in the incubation chamber during incubation to prevent drying of liquid.

Incubate the slide at 37 °C for 60min.

Aspirate the sample.

Wash each well with 50µl of Wash Buffer.

Repeat the washes twice more.

4. Addition of Conjugate

Prepare the conjugate to measure IgG antibodies as shown in the table below:

Number of wells	1X Diluent(µl)	20X Conjugate G (µl) (anti-Human IgG)
2	200	10
4	300	15
6	400	20
8	500	25
10	600	30
12	700	35
14	800	40
16	900	45

Add 50µl of Conjugate G for detection of ENA IgG to the wells as indicated in the layout.

Place the slide in the incubation chamber.

Incubate the slide at 37 °C for 30min.

Aspirate the Conjugate.

Wash each well with 50µl of Wash Buffer.

Repeat the washes twice more.

1 SIMPLE 2 AFFORDABLE 3 ACCURATE

5. Addition of Detection Reagent

Prepare the detection reagent prior to use as shown in the table below:

Number of wells	1X Diluent (µl)	20X Detection (µl) (Streptavidin-HRP)
2	200	10
4	300	15
6	400	20
8	500	25
10	600	30
12	700	35
14	800	40
16	900	45

Add 50µl of the prepared Detection Reagent to each well.

Place the slide in the incubation chamber.

Incubate the slide at 37 °C for 30min.

Aspirate the Detection Reagent.

Wash each well with 50µl of Wash Buffer.

Repeat the washes twice more.

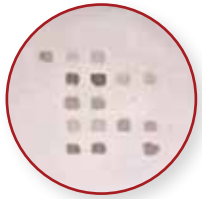
6. Addition of Substrate Solution

Prepare the substrate no more than 30 mins prior to use as shown in the table below:

Number of wells	1X Diluent (µl)	20X Substrate A (µl)
2	200	10
4	300	15
6	400	20
8	500	25
10	600	30
12	700	35
14	800	40
16	900	45

Add 50µl of the diluted Substrate Solution to each array well.

Incubate the slide at room temperature for 5min.



NOTE:

Please monitor color development closely. Brown spots begin to appear in the arrays and the background begins to turn orange.

Allow the reaction to continue longer than 5min based on the signal to noise ratio of spot intensity to background. Incubate the slide for an additional 2 to 3 minutes, if necessary.

An example of a typical array is shown alongside

7. Stopping the Reaction

Aspirate off the Substrate Solution.

Wash each well with 50µl of Wash Buffer to stop the reaction.

Aspirate off the Wash Buffer.

8. Scanning and Analysis

Dry the slide at 37 °C for 30min.

Read the slide following instructions in the “Pictorial© Image Analysis Manual”.

10. Results

Pictorial Image Analysis software measures the color intensity values for each of the array spots.

The intensity values of test spots are corrected against the intensity values from background and control spots using proprietary algorithms.

1. Quality Control

- Control spots in each well are intended to monitor for substantial reagent failure.
- Additional controls may be tested according to guidelines or requirements of local regulatory agencies.
- Measurement of intensity values of Control spots embedded in each PictArray well ensures that the test has been performed correctly.

  The test is valid if the performance control and sample control show acceptable intensity values. If these criteria are not met, the test results are reported as an “Error” by Pictorial software.

2. Units

The test results for patient samples are reported as Internal Unit/ ml defined as the increase in IgG antibody binding to the ENA antigens compared to healthy subjects.

For example, a value of 10IU/ ml is a 10-fold increase in antibody levels compared to non-diseased samples.

The cut-off value of 0.8 IU/ ml was obtained for each antigen by testing serum samples obtained from healthy donors with no detectable antibody binding to ENA antigens on the array.

3. Measuring range

PictArrays generate a semi-quantitative result by measuring the increase in intensity values obtained upon binding of IgG antibodies from patient serum to the ENA antigens.

Negative	less than 0.8 IU/ ml
Borderline	0.81 to 1.4 IU/ ml
Weak Positive	1.41 to 3.5 IU/ ml
Positive	greater than 3.5 IU/ ml

NOTE:

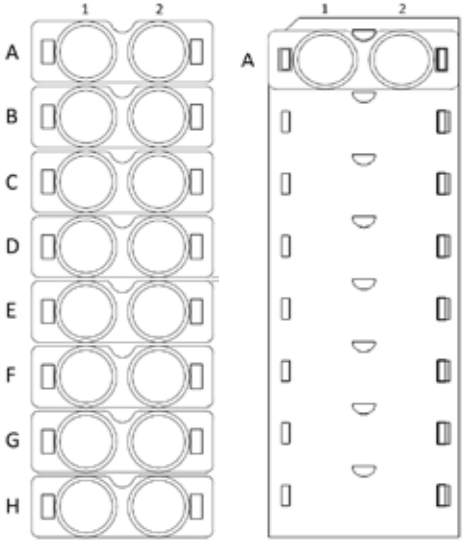
Results reported as borderline (+/-) and weak positive should be interpreted with caution. These results occur when the IU/ ml values are in the grey area which, if the test is repeated, could result in either a positive or a negative outcome.

11. Limitations

- This is a semi-quantitative test and the units when given as IU/ ml are provided as a guide to relative increase in levels compared to a healthy population.
- Repeated freeze-thaw of the sample should be avoided.
- The diagnosis should not be made solely on the basis of PictArray ENA panel test results.
- Test results should be interpreted in conjunction with clinical evaluation and the results of other diagnostic procedures.
- PictArray ENA panel performance has not been tested against lipemic and hemolyzed samples, and those containing high levels of bilirubin.

12. PictArray format

2-well unit layout



PICTORLIMITED

24 Balfour Road, Parnell, Auckland 1052, New Zealand
E info@pictordx.com W www.pictordx.com T +64 9 309 0950
CERTIFIED ISO 9001:2008 ISO 13485