



UltraPlex™ ANA

80 samples 720 TESTS

A multiplexed immunoassay for the qualitative detection of autoantibodies to Sm, SmRNP, RNP-70, Jo-1, Scl-70, SSA, SSB & Centromere B antigens, and the quantitative detection of autoantibodies to dsDNA.

FOR IN VITRO DIAGNOSTIC USE

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Knowledge for Health

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UltraPlex™ ANA User's Guide
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For in vitro diagnostic use only.

Store at 2-8°C upon receipt.

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Intended use

The UltraPlex™ ANA assay is intended for use in the detection of antinuclear antibodies (ANA) in human serum.

Summary and explanation of the assay

The detection of ANA is an important tool in the diagnosis of systemic autoimmune diseases including Systemic Lupus Erythematosus (SLE), Sjörger's Syndrome, Scleroderma, Dermatomyositis and Polymyositis, CREST syndrome and Mixed Connective Tissue Disease (MCTD). Positive detection of ANA in serum suggests the possibility of such diseases. The test results are not diagnostic but are one aspect of diagnostic analysis of individuals. The UltraPlex™ ANA is a multiplexed immunoassay for the qualitative detection of autoantibodies Jo-1, Scl-70, Sm, SmRNP, SSA (60kDa), SSA (52 kDa), SSB, RNP-70, Centromere B and the quantitative detection of antibodies to dsDNA in human serum in one simple assay. The UltraPlex™ SmartStation performs all liquid handling, incubation, tracking of samples and pipettes the completed assay microparticles into a read plate. The UltraPlex™ SmartReader detects, identifies and measures the fluorescence of each UltraCode, enabling the ten immunoassays to be performed simultaneously on a serum sample in a single well.

Principle of the UltraPlex™ ANA assay

The UltraPlex™ ANA system contains pre-formulated SmartBeads (1 per well). Each SmartBead contains the ten autoantigens (Centromere, Jo-1, Scl-70, Sm, SmRNP, SSA (60kDa), SSA (52 kDa), SSB, RNP-70 and dsDNA), each of which is bound to a distinct UltraCode (barcoded microparticle). Details of the antigens are given in Appendix 1.

In addition to the ten autoantigens, an eleventh control UltraCode with no antigen coating is also present (Qmax zero). This enables the monitoring of non-specific binding of samples to the UltraCodes for each patient sample.

Diluted serum samples are incubated with the SmartBeads to allow any antibodies present in the serum to bind to the antigens. After washing away unbound antibodies, the bound antibodies are detected using anti-human IgG fluorescent conjugate. After incubation, excess detection antibody is washed away. The UltraPlex™ SmartReader detects the fluorescence associated with each UltraCode and gives a positive, negative or borderline result for each individual immunoassay, or a figure in IU/ml for anti-dsDNA (10-500 range).

Reagents and materials supplied

ANA ASSAY PLATE One 96 well filter bottom microplate containing 1 SmartBead per well and sealed in a plastic bag with a drying agent. Ready to use.

SAMPLE DILUTION BUFFER (5x) One 20 ml bottle of concentrated solution, containing a detergent and protein in phosphate buffered saline with preservative. Dilute 1 in 5 to a final volume of 100 ml.

WASH BUFFER (10x) One 30 ml bottle of concentrated solution, containing a detergent in phosphate buffered saline with preservative. Dilute 1 in 10 to a final volume of 300 ml.

dsDNA STANDARDS Five vials each containing 300 ìl of diluted human serum. The expected results are given on the control certificate. Ready to use.

NEGATIVE CONTROL One vial containing 300 ìl diluted normal human serum. The expected results are given on the control certificate. Ready to use.

POSITIVE CONTROLS 1 and 2 Two vials each containing 300 ìl of diluted human serum. The expected results are given on the control certificate. Ready to use.

DETECTION ANTIBODY One 20 ml bottle of anti-human IgG dye conjugate in protein-stabilising matrix with a preservative. Ready to use.

DECODING BUFFER One 27 ml bottle of buffer containing glycerol in phosphate buffered saline with a preservative. Ready to use.

READING PLATE One 96 well clear bottom black microtitre plate.

BATCH ID TUBE One vial displaying the batch record information.

Storage and stability

Store the kit at 2–8°C in the original container until use. The reagents are stable until the expiration date on the kit. Do not expose kit to heat, sun or strong light during storage or usage. Do not freeze.

Materials and equipment required

1. Distilled or de-ionised water (>10 M Ω cm) and clean measuring cylinders for the dilution of buffers.
2. UltraPlex™ SmartStation.
3. UltraPlex™ SmartReader.
4. One 96 deep well plate (StarLab Cat# E2796-0501).
5. Four boxes Rainin 1000 μ l tips (Anachem Cat# RT-L1000).
6. Plate sealing tape.
7. Blotting paper (Invitrogen Cat# LC2008).

Warnings and precautions

1. For in vitro diagnostic use only.
2. Not for internal use in humans or animals.
3. Not to be used as a standalone product in making diagnosis, monitoring or screening decisions. The result of this test must not be used for determination of medical intervention.
4. The controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, these reagents should be handled as per local regulations.
5. Optimal results will be obtained by strict adherence to the test protocol.
6. Avoid contact of reagents and samples with the eyes, skin and clothing. Wear disposable gloves, lab clothing and eye protection while handling samples and reagents. Wash hands thoroughly when finished. If contact occurs, wash thoroughly with water or at an eyewash station and consult a physician.
7. The components of this kit are intended for use as an integral unit. The components of different units should not be mixed.
8. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
9. Do not use kit components past the stated expiry dates. Avoid microbial contamination of all reagents and samples or incorrect results may occur.
10. The procedure is for running 80 tests simultaneously rather than in small batches.

Specimen collection and handling

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. If frozen, ensure samples are thoroughly defrosted and mixed before use.
3. Avoid repetitive freezing and thawing of serum samples. Lipaemic, haemolysed or contaminated samples may give poor results and should not be tested.
4. A minimum sample volume of 100 µl is required for the UltraPlex™ SmartStation.
5. Up to 80 serum samples can be tested per run.
6. Samples must be contained in tubes compatible with the UltraPlex™ SmartStation sample racks.

Preparation for the assay

1. Bring all specimens and kit reagents to room temperature (18-25°C) and mix gently.
2. Dilute contents of wash buffer concentrate 10x to 300 ml with distilled or de-ionised water in a suitable storage container.
3. Dilute contents of sample dilution buffer concentrate 5x to 100 ml with distilled or de-ionised water in a suitable storage container.
4. Briefly invert to mix and then centrifuge the tubes containing the dsDNA standards and controls to ensure the liquid is at the bottom of the tubes.
5. Ensure that the samples are fully mixed, especially if they have been stored for a long period of time.
6. It is important to not introduce air bubbles into either the controls or samples during any mixing.

UltraPlex™ ANA assay procedure

The assay procedure is composed of two elements. Firstly, the UltraPlex™ SmartStation robotic liquid handling system performs all liquid handling, incubation, tracking of samples and then pipettes the completed assay microparticles into a read plate. Secondly, the UltraPlex™ SmartReader detects, identifies and measures the fluorescence of each UltraCode and provides the data output.

Separate operating manuals describe the operation of the UltraPlex™ SmartStation and SmartReader, and should be read thoroughly in conjunction with this user guide. Those operating manuals describe in detail the set-up, maintenance and shut-down procedures of the UltraPlex™ SmartStation and SmartReader.

The aspects of the running of the UltraPlex™ SmartStation that are specific to the UltraPlex™ ANA assay are described below:

1. The UltraPlex™ ANA assay is designed to test 80 samples on a single assay plate. Load these samples into positions 1 – 80 of sample racks 1 – 5 (from back to front in rack 1, then rack 2, etc.).
2. Load the controls and standards provided onto the sixth sample rack of the UltraPlex™ SmartStation in the following order:
 1. Negative control
 2. Positive control 1
 3. Positive control 2
 4. dsDNA standard 1
 5. dsDNA standard 2
 6. dsDNA standard 3
 7. dsDNA standard 4
 8. dsDNA standard 5

3. Load the batch number tube in position 9 of sample rack 6.
4. Load the sample racks onto the UltraPlex™ SmartStation deck in the order rack 1 to rack 6 from left to right.
5. Load the reagent troughs as follows:
 - T1: 50 ml of diluted sample dilution buffer
 - T2: 50 ml of diluted sample dilution buffer
 - T3: All of provided detection antibody (20 ml)
 - T4: All of provided decoding buffer (27 ml)

The remainder of the set-up of the UltraPlex™ SmartStation is the same for all UltraPlex™ assays, and is detailed in the operating instructions for the SmartStation. For convenience they are also provided in summary form below and in the quick start guide.

1. Place diluted wash buffer in the wash buffer trough.
2. Load four full tip boxes on the deck.
3. Place a fresh piece of blotting paper on the blotting block.
4. Load the deep well dilution plate, read plate and assay plate onto the deck in their respective positions, with well A1 at the back left in each case.

The assay can now be started using the **UltraPlex™ ANA** process in the Lirix software, as described in the UltraPlex™ SmartStation operating manual.

Following completion of the liquid handling steps seal the UltraPlex™ ANA read plate using plate sealing adhesive film and transfer the plate to the UltraPlex™ SmartReader system for detection, identification and measurement of the fluorescence of each UltraCode. This procedure is detailed in the UltraPlex™ SmartReader operating manual.

Interpretation of the results

The UltraPlex™ SmartDecode software determines whether the samples are negative, borderline or positive for autoantibodies to Jo-1, Scl-70, Sm, SmRNP, SSA (60 kDa), SSA (52 kDa), SSB, RNP-70, and Centromere B.

It also returns a quantitative result for dsDNA in IU/ml using a standard curve. The dsDNA assay is calibrated in IU/ml against the WHO international reference serum Wo80¹.

From studies of samples from healthy blood donors we have established the following guidelines for interpretation of the dsDNA data:

	Anti-dsDNA antibody (IU/ml)
Normal	<40
Borderline	40 - 50
Elevated	>50

Quality control

In order for the assay to be valid, the following criteria should be met:

- Standards and positive and negative controls must be included in each assay run.
- The values obtained for all the controls should be in the ranges specified on the QC certificate.
- The curve shape should be similar to the standard curve shown in the QC certificate.

If any of the above criteria are not met, the assay is invalid and should be repeated.

Performance characteristics

Precision

Intra-assay precision (within an assay) was calculated for each sample from the results of five replicates in a single assay.

Inter-assay precision (between assays) was calculated for each sample from the results of five different assays with a duplicate of each sample on each assay.

The results shown are coefficients of variation of the fluorescence intensities of the specific UltraCodes following subtraction of the Q_{max} zero UltraCode (except in the case of dsDNA for which values stated are in IU/ml).

Antigen	CV (%)
Centromere B	14.3%
SSA (60 kDa)	19.9%
SSA (52 kDa)	9.0%
SSB	8.5%
Scl-70	8.3%
Jo-1	9.1%
Sm	3.5%
SmRNP	8.9%
RNP-70	15.7%
dsDNA*	11.3%

Table 1. Intra-assay precision data

*values in IU/ml

Antigen	CV (%)
Centromere B	7.9%
SSA (60 kDa)	5.7%
SSA (52 kDa)	5.7%
SSB (La)	3.3%
Scl-70	6.7%
Jo-1	16.4%
Sm	13.9%
SmRNP	9.7%
RNP-70	12.3%
dsDNA*	20.7%

Table 2. Inter-assay precision data

*values in IU/ml

Linearity of the dsDNA assay

To assess the linearity of the dsDNA assay, five samples, containing varying concentrations of anti-dsDNA autoantibodies, were serially diluted with sample dilution buffer to produce a range of values within the dynamic range of the assay.

The samples showed linearity across the full range of the assay, with dilutions of the five samples from 1:50 to 1:8,000. The mean of observed over expected values was 108% (range 87 to 125%).

Sensitivity of the dsDNA assay

Sensitivity was calculated from the combined results of two studies. Firstly, eight replicates of three samples (negative for anti-dsDNA autoantibodies) were analysed in a single assay. Secondly, 56 samples from healthy blood donors were analysed for their levels of anti-dsDNA.

The sensitivity of the assay was determined to be <10 IU/ml.

Warranty

The UltraPlex™ ANA assay is warranted to perform as described in this packaging insert. Any change or modification to the procedure as described by Pronostics Ltd. may affect the results and is not recommended. Pronostics Ltd. disclaims any implied warranty of merchantability or fitness for a particular use, and in no event will Pronostics Ltd. be liable for consequential or indirect damages.

References

Feltkamp T E W. *et al.* Annals of Rheumatic Diseases. 1988; 47: 740-746.

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Appendix 1. Details of autoantigens

Antigen	Description	Purity
Centromere B	Recombinant*	>90 % by SDS-PAGE
SSA (60 kDa)	Native, purified from bovine thymus.	>90 % by SDS-PAGE
SSA (52 kDa)	Recombinant*	>90 % by SDS-PAGE
SSB	Recombinant*	>90 % by SDS-PAGE
Scl-70	Recombinant*	>80 % by SDS-PAGE
Jo-1	Recombinant*	>90 % by SDS-PAGE
Sm	Native, purified from bovine thymus.	>90 % by SDS-PAGE
SmRNP	Native, purified from bovine thymus.	>90 % by SDS-PAGE
RNP-70	Recombinant. Expressed in <i>E. coli</i> bacterial cells.	>90 % by SDS-PAGE
dsDNA	Bacterial plasmid molecule (Size 3,400 base pairs). Propagated in and purified from <i>E. coli</i> .	> 95 % closed-circular form

*Expressed by recombinant baculovirus infection of *Spodoptera frugiperda* Sf9 insect cells.



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