

Information

Chip Incubation. For convenience and high signal reliability, slide incubation is best performed using a CapitalBio BioMixer™ II Microarray Hybridization Station (Cat. No. 120030) which helps to reduced edge-effects.



CapitalBio Antinuclear Antibody Profile Test Chip Kit

Cat. No. 303010

User Manual

For Laboratory Research Use Only
Not for Diagnostic Purposes

CapitalBio Corporation

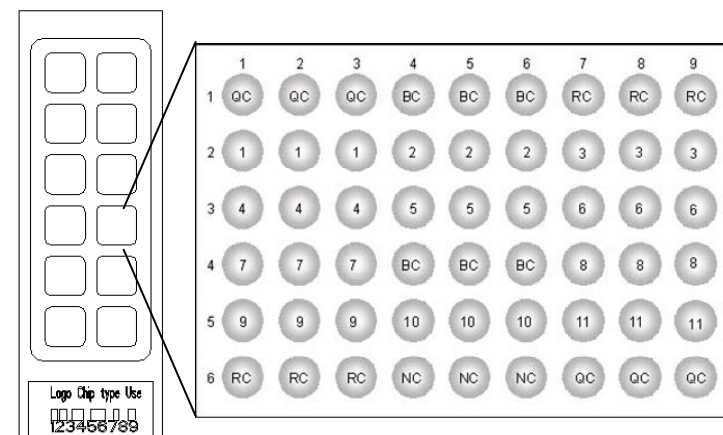
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| Groups | Autoantibody | Frequency (%) | |
|--|-----------------------|---------------|--------------------|
| | | US Population | Chinese Population |
| Systemic Lupus Erythematosus (SLE) | SSA | 20-50 | 20-60 |
| | SSB | 10-20 | 10-24 |
| | Sm | 10-50 | 20-47 |
| | RNP 68 | 20-50 | 10-40 |
| | dsDNA | 25-85 | 39-90 |
| | Ribosomal P0 | 10-20 | 8-27 |
| Sjögrens-syndrome (SS) | ANA | 90-100 | 80-100 |
| | SSA | 40-90 | 41-95 |
| | SSB | 16-60 | 18-80 |
| Systemic Scleroderma (SSc) | ANA | 40-85 | 60-80 |
| | Scl-70 | 20-75 | 18-60 |
| Polymyositis/Dermatomyositis | ANA | 15-90 | 40-98 |
| | Jo-1 | 25-40 | 20-30 |
| Mixed Connective Tissue Disease (MCTD) | ANA | 30-80 | 40-60 |
| | RNP 68 | 95-100 | 90-100 |
| Raynaud's phenomenon | ANA | 70-100 | 95-100 |
| | Centromere B | 15-20 | 8-58 |
| CREST syndrome | ANA | 20-60 | 22 |
| | Centromere B | 49-96 | 38-90 |
| Normal/healthy | ANA | 60-90 | 40-98 |
| | SSA | <2 | 0 |
| | SSB | <1 | 0 |
| | Sm | 0 | 0 |
| | RNP 68 | 0 | 0 |
| | Scl-70 | 0 | 0 |
| | Jo-1 | 0 | 0 |
| | Centromere B | <1 | 0 |
| | dsDNA | <1 | 0 |
| | Ribosomal P0 | 0 | 0 |
| ANA | 3-12 (Titer<1:160) | <5 | |

Table 4. Characteristics of anti-nuclear antibodies in the US population and the Chinese population [1-20]

- 3.2 Wash buffer (10× concentrate), 1 vial, 50 ml. Phosphate saline buffer with 0.1% Tween-20.
- 3.3 Sample buffer (1× concentrate), 1 vial, 15 ml. Phosphate saline buffer with 1% bovine serum albumin.
- 3.4 Cyanine 3-conjugated goat anti-human IgG (10× concentrate), 1 vial, 220 μl.
- 3.5 Human positive control serum (10× concentrate), 1 vial, 50 μl.
- 3.6 Human negative control serum (10× concentrate), 1 vial, 50 μl.



QC: Quality Control BC: Blank Control
 RC: Reaction Control NC: Negative Control
 Antigens: 1: Jo-1 2: Sm 3: Scl-70 4: CENP-B
 5: dsDNA 6: SSB 7: SSA-52 8: Extracts of Hep-2 cells
 9: SSA-60 10: Ribosomal P0 11: RNP-68

Figure 1. ANA Chip layout. The left six blocks marked as A1-A6, and the right six blocks marked as B1-B6. Each block contains 11 antigens and 4 controls.

4. Storage and stability

The kit should be stored at 2-8°C. Do not freeze.

The kit remains stable for at least 6 months from date of manufacture if handled properly. Do not use the kit after the stated expiry date. If the vials are opened, the kit should be used within one month. The kit is stable during shipment under recommended shipping temperature conditions (2-8°C).

Table 2. Cutoff Values of each antigen

| Antibody | Cutoff Value | |
|---------------------------|--------------|----------|
| | Negative | Positive |
| Anti-SSA-52 ^{*1} | <1.00 | ≥1.00 |
| Anti-SSA-60 ^{*1} | <1.00 | ≥1.00 |
| Anti-SSB | <1.00 | ≥1.00 |
| Anti-Sm | <1.00 | ≥1.00 |
| Anti-RNP-68 ^{*2} | <1.00 | ≥1.00 |
| Anti-Scl-70 | <1.00 | ≥1.00 |
| Anti-Jo-1 | <1.00 | ≥1.00 |
| Anti- Centromere-B | <1.00 | ≥1.00 |
| Anti-dsDNA | <1.00 | ≥1.00 |
| Anti- Ribosomal P0 | <1.00 | ≥1.00 |
| ANA | <1.00 | ≥1.00 |

*1 The SSA-52 and 60 KD versions of SSA are reported as one antigen (SSA).

*2 RNP-68 in the test kit is the recombinant RNP-68 antigen (not the Sm/RNP complex). The autoantibody detected is RNP-68 only.

11. Expected values

Clinical studies were undertaken with both normal and patient populations of Chinese ethnicity, with females between the ages of 19-77 and males between the ages of 20-76.

Current literature reporting the prevalence of antigen positivity/ negativity within several US patient samples is listed in **Table 4**^[1-12]. No information regarding the prevalence within specific ethnic populations in the US was included. A comparison of the US and Chinese populations demonstrates a difference in the prevalence with some antigens, but overall both show a similar trend. The higher frequency of SSB amongst the Chinese population sample may indicate ethnicity differences.

7. Specimen collection, storage and handling

- 7.1 Blood specimens should be collected by a certified medical technologist. Serum is separated by centrifugation at 3000 rpm for 5 min.
- 7.2 Serum should be clear and non-hemolyzed.
- 7.3 Specimens can be refrigerated at 2-8°C for up to five days, or stored at -20°C for up to six months.
- 7.4 Avoid repetitive freezing and thawing of serum samples.

8. Assay procedure

8.1 Pre-test preparation

- 8.1.1 Remove the individual components from storage and allow them to warm to room temperature (20-25°C). One chip can test up to 10 specimens. It is mandatory to include one Negative Control and one Positive Control for each individual chip used.
- 8.1.2 Wash buffer: The wash buffer included is in 10× concentrate format. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting.
For one chip, measure 12 ml of concentrate and put it into a 250 ml bottle. Measure 108 ml of distilled water into the 250 ml bottle to make up a final volume of 120 ml. Mix well by stirring or gentle shaking before use.
- 8.1.3 Sample buffer: ready to use.
- 8.1.4 Conjugate: The conjugate is a 10×concentrate solution. For each chip used, 50 µl of the 10× concentrate solution is diluted with 450 µl of sample buffer in a small test tube. Mix the conjugate well by gently pipetting. Cover it with aluminum foil to avoid light if the conjugate is not used immediately.
- 8.1.5 Human positive and negative control serum: The serum controls are 10× concentrates.
For one chip, add 10 µl of concentrate and 90 µl of sample buffer in one tube. Mix well by pipetting.
- 8.1.6 Specimen: Dilute the specimen 100 fold before use.
- 8.1.7 Recommended procedure: Mix 10 µl of serum with 90 µl of sample buffer in a microplate well. Then transfer 10 µl of the 1:10 mixture with 90 µl of sample buffer into a new well to make the 100-fold final sample dilution. For assay accuracy, it is important that the sample is thoroughly mixed at each dilution step.

1. Introduction

The Antinuclear Antibody Profile Test Chip Kit (ANA Chip Kit) is a protein chip immunoassay kit intended for the semi-quantitative detection of antinuclear antibody (ANA), and the other nine individual antibodies against SSA, SSB, Sm, RNP 68, Scl-70, Jo-1, centromere B, dsDNA and Ribosomal P0, respectively in human serum. The presence of ANA or other autoantibodies can be used in conjunction with clinical symptoms and other laboratory findings to assist physicians in the diagnosis of particular types of systemic autoimmune diseases, including Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD), Sjögren's syndrome (SS), Systemic Sclerosis (SSc), Dermatomyositis, Polymyositis, CREST syndrome, and Raynaud's phenomenon. The test results from this kit serve only as a reference for clinicians when a diagnosis is made. A diagnosis cannot be made based only on the results obtained from the test kits.

2. Principle of the Test

The ANA Chip Kit is designed to detect the IgG class autoantibodies in human sera. The test kit is based on microarray immunoassay. Purified antigens (SSA 52, SSA 60, SSB, Sm, RNP 68, Scl-70, Jo-1, dsDNA, centromere B, Ribosomal P0) and extracts of Hep-2 cells are immobilized on the chip surface to serve as an antigenic substrate. Dilutions of the patient serum samples are added to the chip surface and incubated, allowing specific antibodies that exist in the serum sample to bind to its corresponding antigen spotted on the chip surface. Unbound antibody molecules and other serum proteins are removed by subsequent washing steps. An anti-human IgG labeled with Cy3 fluorescence dye is then applied to the chip surface and incubated. Unbound conjugates are washed off. The fluorescent signals that remain on the chip surface are quantified by a microarray scanner. The relative titer of the detected antibodies in human serum can be assessed semi-quantitatively through the normalized values of the measured fluorescence signal intensities. The software of the ANA test system is then used to analyze the data obtained from the scanner and to generate the test reports.

3. Contents of the Kit

3.1 Antinuclear antibody profile test chip, (4 chips, individually packaged in a chip centrifuge tube). Each chip contains twelve individual blocks, and each block contains 54 spots which are antigens or controls (**Figure 1**).

References

- 1 Kavanaugh A, Tomar R, Reveille J, et al. Guidelines for clinical use of the antinuclear antibody test and tests for specific auto antibodies to nuclear antigens. *Arch Pathol Lab Med*, 2000, 124:71-81.
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5. Warnings and precautions

- The ANA chip kit is for *in vitro* diagnostic use only.
- Human sera used for this product have been tested using FDA approved methods and found to be negative for antibodies to HIV-1, HIV-2, HBsAg and HCV.
- Do not interchange kit contents from different lots.
- Incomplete or inadequate washing of the chip may cause high background or false positive results.
- Do not use unused blocks on a used chip, as it may cause cross-contamination.
- The conjugate component is light sensitive. Do not expose this reagent to strong light sources.
- Bring all reagents and specimens to room temperature (20-25°C) prior to use. Store the unused reagents at 2-8°C immediately after use.
- Wear disposable gloves while handling specimens or kit reagents, and wash hands thoroughly afterwards.
- Do not pipette samples and reagents by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- The wash buffer, sample buffer, controls, and conjugate contain aseptic Proclin 300 at a concentration of 0.1% (v/v). Avoid contact with the skin.

6. Materials required

6.1 Additional Materials required for the assay, but not supplied in this kit:

- Micropipettes for 10 µl, 100 µl and 1000 µl
- Clean pipette tips matched to the 10 µl, 100 µl and 1000 µl pipettes
- ELISA microwell plate (12×8 wells)

6.2 Equipment required

- LuxScan 10K-B Microarray Scanner (CapitalBio, Cat. No. 100020)
- Vortex mixer
- Centrifuge (>3000 rpm)
- Laboratory timer
- Incubation cassette (CapitalBio, Cat. No. 430020)

6.3 Preparation of reagents

- Distilled or deionized water
- Graduated cylinders: 25 and 200 ml
- Reagent bottles: 250 and 500 ml

Table 3. Characteristics of anti-nuclear antibodies in the Chinese population^[13-20]

| Disease Groups | Frequency (%) | | | | |
|--------------------|---------------|-------|-------|--------|--------|
| | SSA | SSB | Sm | RNP | Sci-70 |
| SLE | 20-60 | 10-24 | 20-47 | 10-40 | 0 |
| SS | 41-95 | 18-80 | 0 | - | 0 |
| SSc | 17 | 10 | 0 | 5-20 | 18-60 |
| PM/DM | 18-22 | 22 | 0 | 20 | 0 |
| MCTD | 22 | 5 | 0 | 90-100 | 0 |
| Raynaud phenomenon | - | - | 0 | - | 0 |
| CREST Syndrome | 10-17 | 10 | 0 | 5-20 | 0 |
| Normal/healthy | 0 | 0 | 0 | 0 | 0 |

| Disease Groups | Frequency (%) | | | | |
|--------------------|---------------|--------------|-------|------|--------|
| | Jo-1 | Centromere B | dsDNA | rRNP | ANA |
| SLE | 0 | 2-9 | 39-90 | 8-27 | 80-100 |
| SS | 0 | 14-22 | 0 | 15 | 60-80 |
| SSc | 0 | 16-53 | 0 | 17 | 40-98 |
| PM/DM | 20-30 | - | 0 | - | 40-60 |
| MCTD | 0 | - | 0 | 7 | 95-100 |
| Raynaud phenomenon | 0 | 8-58 | 0 | - | 22 |
| CREST Syndrome | 0 | 38-90 | 0 | 17 | 40-98 |
| Normal/healthy | 0 | 0 | 0 | 0 | <5 |

12. Limitations of the procedure

Patients with specific autoimmune disease may not be positive for autoantibodies for a specific antigen(s). A definitive clinical diagnosis should be made by the physician, based on clinical findings and on comprehensive laboratory examinations.

This assay is only validated for analysis on the LuxScan 10K-B Microarray Scanner.

13. Interfering substances

No interference has been observed with hemolytic and lipidemic serum. However, for practical reasons it is recommended that grossly hemolyzed or lipidemic samples should be avoided.

No cross-reactivity or interference was seen due to complement, anti-histone antibodies and hypergammaglobulemia for each antigen on the chip, indicating that there is no cross interference with the ANA chip kit antigens.

No significant interference to the assay was detected when methylprednisolone, dexamethasone or methotrexate were added separately to serum samples at concentrations of 100 µg/ml or 500 µg/ml.

8.2 Immunoreaction procedure

- 8.2.1 Determine the total number of chips needed and remove the appropriate number of chips from the package.
- 8.2.2 Place a protein chip incubation cassette on a level table (Note: the side marked with "A, B, C, D" should face toward the operator), and fill the reservoir well in the base with a max of 300 µl distilled water to keep the chamber humid. Place the chip into the cassette with the barcoded side facing up and the barcode towards the operator.
- 8.2.3 Transfer 30 µl of each of the diluted controls and specimens from the dilution plate with a multi-channel pipette or a repeating pipette to the chip surface. Samples should be added in blocks A2-A6 and B2-B6. The positive serum control should be added in block A1. The negative serum control should be added in block B1.
- 8.2.4 Cover the protein chip incubation cassette and incubate at room temperature (20-25°C) for 30 minutes.

8.2.5 Washing

- 8.2.5.1 Place the chip in a chip centrifuge tube filled with 25 ml of wash buffer. The tube is then capped and shaken for 10 times up and down. Take out the chip and empty the tube.
- 8.2.5.2 Refill the tube with 25 ml of wash buffer and shake for 5 minutes at 150 rpm on the shaker with the tube side marked with "CapitalBio" facing up.
- 8.2.5.3 Remove the chip and empty the tube contents. Replace the chip back into the tube and centrifuge for 1 minute at 1000 rpm to dry the chip.

8.2.6 Conjugate incubation

- 8.2.6.1 Place the chip into the protein chip incubation cassette as described in 8.2.2. Add 30 µl of diluted conjugate in each block with a multi-channel pipette.
- 8.2.6.2 Cover the incubation cassette and incubate at room temperature (20-25°C) for 30 minutes.

8.2.7 Washing

Wash the chip again using the procedure described in section 8.2.5. The chip is then ready for scanning.

8.3 Data Acquisition

After the completion of the conjugate incubation step, the chip should be stored at room temperature and analyzed as soon as possible to avoid fluorescence quenching. Place the chip into the LuxScan 10K-B Microarray Scanner chamber, and generate the result according to the "ANA Test System" instructions. If the chip cannot be analyzed immediately, it should be stored in dark and dry condition, and analyzed within 24 hours.

9. Quality control

The positive control and negative control should be run with every chip to ensure that all reagents and procedures performed properly. Expected values for each control are shown in **Table 1**.

Table 1. Expected values for quality controls

| Control | Quality Control |
|---------|-----------------|
| BC | ≤500 |
| NC | ≤500 |
| QC | ≥3000 |
| RC | ≥6000 |

10. Interpretation of results

The purpose of the Antinuclear Antibody Test Chip Kit is to assist physicians to make a clinical diagnosis for defined systemic autoimmune diseases.

Positive: A positive result is the calculated number of one antigen ≥ 1.0 .

Negative: A negative result is the calculated number of one antigen < 1.0

Physicians should pay particular attention to samples if the calculated number ranges between 0.6 and 1.0, which is regarded as the uncertainty range. If an autoimmune disease is suspected, one or more additional analysis methods are recommended to be used at either the same or at different titers to confirm the test results.