

AESKUBLOT ANA-12 Pro

Ref 4000



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| Product Ref. | 4000 |
| Product Desc. | ANA-12 Pro |
| Manual Rev. No. | 005 : 2013-02-28 |

Instruction Manual

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1 Intended Use

AESKUBLOT ANA-12 Pro is a membrane based enzyme immunoassay for qualitative detection of IgG antibodies against nucleosomes, dsDNA, histones, SmD1, U1-snRNP, SS-A/Ro60kD, SS-A/Ro52kD, SS-B/La, SCL-70, CENP-B, Jo-1 and ribosomal P-protein (Rib-P0) in human serum or plasma. Antigens are located as parallel lines at exactly defined positions on a nitrocellulose membrane.

The assay is a tool in differential diagnosis of systemic rheumatic diseases.

2 Clinical Application and Principle of the Test

Anti-nuclear antibodies (ANAs) are an important tool for the differential diagnosis of systemic rheumatic diseases. An indirect immunofluorescence test (IFT) on eucaryotic cells like HeLa has been the established method for the detection of ANAs. Single antibody specificities are distinguished by fluorescence patterns. However, a more specific testing method by Line Immuno-Assays (LIAs) employing the target antigens is also available for a simple and reliable differentiation of ANAs.

ANAs are especially found in active and inactive systemic lupus erythematosus (SLE), mixed connective tissue diseases (MCTD), scleroderma, Sjögren's syndrome and polymyositis.

Antibodies against:

- Nucleosomes are directed against epitopes of the histone complex (nucleosome). In addition, anti-dsDNA and anti-histone antibodies can recognize epitopes of the nucleosome. In comparison to anti-dsDNA antibodies, anti-nucleosome antibodies are more sensitive and can provide a useful addition to the diagnosis of SLE (Chabre et al. 1995; Bruns et.al. 2000). Furthermore, they have pathogenetic significance in lupus nephritis (Van Bruggen et al. 1996; Amoura et al. 1999).
- dsDNA are regarded as being specific for SLE and have been observed in approximately 50-80 % of the patients.
- Histones are common in SLE patients. However they also occur in other connective tissue diseases. Antibodies to histones in the absence of other autoantibodies (especially anti-dsDNA) are a characteristic marker for drug-induced lupus erythematosus (Rubin 1999).
- SmD1 (Smith antigen) are directed against the core protein D1 of small nuclear ribonucleoproteins (snRNPs). Anti-Sm-antibodies as well as antibodies against double stranded DNA (dsDNA) are highly specific for SLE and thus are included in diagnostic and classification criteria for SLE.
- U1-snRNP are pathognomonic for MCTD but do also occur in SLE. A high titer of antibodies against this antigen is typical for the sharp's syndrome.
- SS-A (Ro; soluble cytoplasmic and/or nuclear ribonucleoproteins of 52 kDa and 60 kDa) and antibodies against SS-B (La; 48 kDa protein associated with RNA-polymerase III) are mainly found in high titers for primary and secondary Sjögren's syndrome but also in SLE, congenital heart block and neonatal lupus.
- Scl-70 are directed against DNA-topoisomerase I. They are highly specific for systemic scleroderma and are indicative of a severe course of the disease.
- CENP-B (80 kDa centromere protein B) are typical for the CREST-syndrome (69 % of CREST patients), which is a more protracted type of systemic sclerosis.
- Jo-1 are directed against histidyl-tRNA-synthetase (a cytoplasmic protein involved in protein biosynthesis) and are found in 20-40 % of patients with polymyositis and dermatomyositis.
- ribosomal P-proteins are directed against several phosphoproteins of the large ribosomal subunit. They occur in patients with systemic lupus erythematosus (Elkon et al. 1985) and in lupus patients with cerebral involvement (Bonfa et al. 1987).

Principle of the test

The antigens are applied as lines on a nitrocellulose membrane. The membrane is blocked to prevent unspecific reactions. Membrane-strips with specific antigens at exactly defined positions are incubated in serum/plasma samples diluted 1:101. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples. Unbound conjugate is washed off in the following step. After the addition of the TMB-substrate it is converted by an enzymatic reaction to a blue precipitate. The reaction is stopped by distilled water.

3 Kit Contents

| TO BE RECONSTITUTED | | | | |
|---|--------------------------------|-----------------------|----------------|--|
| Item | Quantity | Cap color | Solution color | Description / Contents |
| Blocking Reagent | 3 x for 10 ml Concentrate each | white | N/A | Non-fat dry milk powder for preparation of 3 x 10 ml sample buffer |
| Wash Buffer (20x) | 1 x 50 ml | white | colorless | 20x concentrated for preparation of 1 L Tris buffer, pH 6.9 ± 0.2 |
| READY TO USE | | | | |
| Item | Quantity | Cap color | Solution color | Description / Contents |
| Conjugate, IgG | 1 x 10 ml | blue | colorless | Anti-human immunoglobulin G (IgG) conjugated to horseradish peroxidase |
| TMB Substrate | 1 x 10 ml | black | colorless | Stabilized TMB/H ₂ O ₂ |
| Membrane strips | 24 strips | colour coding: yellow | N/A | Coated antigens see Intended use |
| tweezers, reference template, scoring sheet, adhesive strip (double-sides, black) | 1 pcs. each | N/A | N/A | N/A |
| incubation tray | 3 pcs. | N/A | N/A | N/A |
| Labels for sample buffer | 3 pcs. | N/A | N/A | N/A |
| MATERIALS REQUIRED, BUT NOT PROVIDED | | | | |
| rocking platform, cylinder 1000 ml, pipette or cylinder for 10 ml, precision pipettes (10, 1000 µl), absorbent or filter paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.). | | | | |

4 Storage and Shelf Life

Store all reagents and membrane-strips at 2-8°C/35-46°F in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for at least six weeks. Reagents and strips shall be used within the expiry date indicated on each respective component. Don't use components after the expiry dates. Avoid intense exposure of TMB solution to the light.



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5 Precautions of Use and General Introductions

5.1 Health hazard data

This product is for IN VITRO DIAGNOSTIC use only. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous under the conditions of intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend avoiding contact with eyes and skin and wearing disposable gloves.

Substrate contains kathon (1% v/v) as preservative. It must not be swallowed or allowed to come into contact skin or mucous membrane.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

Handle patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

To differentiate between the various **AESKUBLOT**-tests available, a color coding is applied above the reference line of the strips:

| Colour coding | AESKUBLOT |
|---------------|---------------------------|
| yellow | ANA-12 Pro |
| orange | ANA-17 Pro |
| blue | Myositis Pro |
| brown | Liver Pro |
| purple | Vasculitis Pro |
| black | Gastro Pro |
| green | Borrelia-G and Borrelia-M |

In case that the product information, including the labeling, is incorrect please contact the manufacturer or the supplier of the test kit.

Blocking Reagent and wash buffer may be interchanged between lots and test kits. All other components are specific for each test kit and are not to be interchanged. Do not exchange reagent components between autoimmunity and borrelia diagnostic tests!

For handling of conjugate do not use polystyrene vessels.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/ 98.6°F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips priorly used with other reagents.

The intensity of the band colour does not necessarily correlate with antibody titers obtained by other reference methodologies.

Samples from apparent normal blood donors may contain autoantibodies.

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If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum/plasma samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum/plasma samples should be used during the first 8 h. Alternatively, the samples should be stored in tightly closed vials at 2-8 °C/35-46 °F for up to 48 h, or frozen at -20 °C/-4 °F for longer periods. Avoid repeated thawing and freezing. Do not use heat inactivated samples.

7 Assay Procedure

7.1 Preparations prior to starting

Confirm that no salt crystals have been formed in the concentrate. If this happened, dissolve the crystals by slightly warming, room temperature should be enough, the concentrate. Dilute concentrated wash buffer 1:20 with distilled water (e.g. 950 ml plus 50 ml).

For preparation of sample buffer: add 10 ml wash buffer to one bottle Blocking Reagent and mix well.

7.2 Test Steps

Important notes:

Follow exactly this protocol. Make sure that the two components mentioned in the protocol are added to the tray in steps 2, 6, 9.

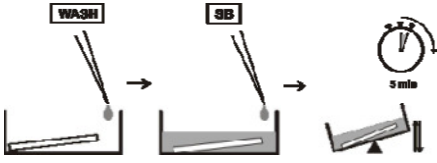
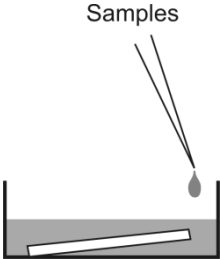
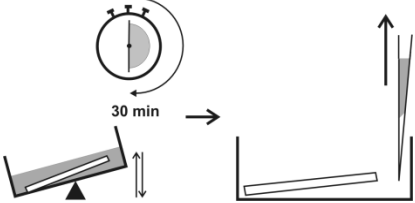
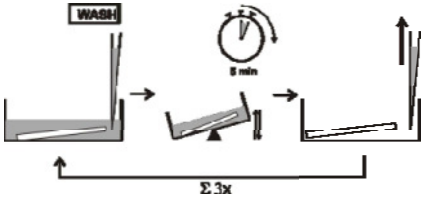
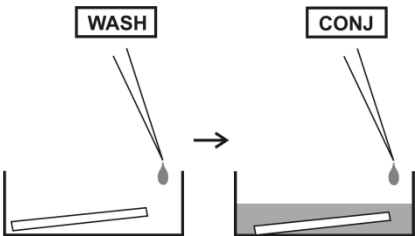
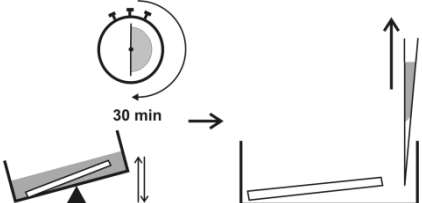
Do not let strip dry out during incubation steps.

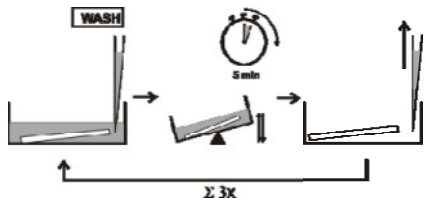
Do not touch strip with fingers, use tweezers.

Remove diluted samples completely after incubation of strip to avoid carry over.

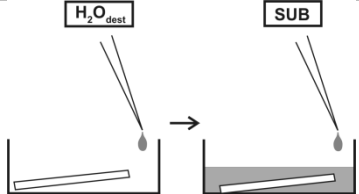
Continuously shake strip during incubation steps.

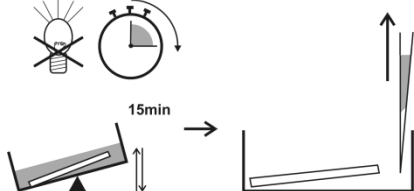
Give sample buffer, conjugate and substrate together with the wash buffer to one side of the incubation tray. Do not allow to flow over the strip.

| Step | Description |
|-------------------------------|---|
| 1. | Ensure the preparations, from step 7.1 above, have been carried out prior to test begin. |
| 2. |  <p>Put strip in correct orientation into incubation tray (reference line and colour coding upwards). Put 700 µl wash buffer and 300 µl sample buffer in the incubation tray. Moisten strip with the solution and incubate for 5 minutes with agitation.</p> |
| CONTROLS & SAMPLES | |
| 3. |  <p>Pipette 10 µl serum/plasma sample into the designated incubation trays with sample buffer.</p> |
| 4. |  <p>Incubate for 30 minutes at 20-32°C/68-89.6°F with agitation. After that remove sample completely.</p> |
| 5. |  <p>Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.</p> |
| CONJUGATE | |
| 6. |  <p>Pipette 700 µl wash buffer and 300 µl conjugate into each incubation tray with strip.</p> |
| 7. |  <p>Incubate for 30 minutes at 20-32°C/68-89.6°F with agitation. Remove conjugate.</p> |

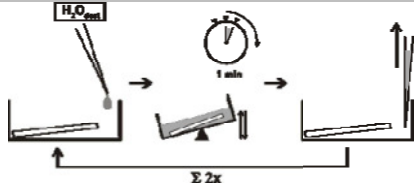
8.  Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.

SUBSTRATE

9.  Pipette 700 µl dH₂O and 300 µl substrate into each incubation tray with strip.

10.  Incubate for 15 minutes at 20-32°C/68-89.6°F with agitation, protected from intense light. Remove substrate.

STOP

11.  Pipette 2 ml dH₂O into each incubation tray with strip. Incubate 1 minute with agitation. Remove dH₂O. Repeat this step one time.

12. Remove strip of the incubation tray. Dry strip between filter paper

13. Analyze results within 24 h.



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8 Qualitative Interpretation

8.1 Manual Analysis

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Colour intensity of cut-off control is weaker than colour intensity of functional control

Fix dried strip onto scoring sheet aligned with reference line. Align reference template with the strip reference line. Interpret results only in reference to cut-off control of each strip.

Each test kit contains a colour copy with all bands provable in the test.

The analysis is carried out by means of comparing the colour intensities of the bands with colour intensity of the cut-off control. The test is equivocal if the intensities do not significant differ. Is the colour more intensive the test result is positive, if the colour intensity is weaker, the test is negative.

The results can be recorded on the scoring sheet.

In case that the values of the controls do not meet the criteria, the test is invalid and has to be repeated. We recommend retesting samples that are borderline.

The following technical issues should as well be checked: expiry date of (prepared) reagents, storage conditions, pipettes, equipment, incubation conditions and washing methods.

If the samples tested show aberrant values or any kind of deviation or if the validation criteria are not met because of reasons outside the operator's responsibility, please contact the manufacturer or the supplier of the test kit.

Medical laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as stated in national regulations.

9 Technical Data

| | |
|---------------------------|--|
| Sample material: | serum or plasma |
| Sample volume: | 10 µl of sample |
| Total incubation time: | 112 minutes at 20-32°C/68-89.6°F |
| Storage: | at 2-8°C/35-46°F; use original vials only. |
| Number of determinations: | 24 tests |

10 Performance Data

10.1 Relative Sensitivity and Specificity

In order to determine the positive agreement (relative sensitivity), 85 sera from IIF antibody-positive patients were tested in **AESKUBLOT ANA-12 Pro**. For determination of the negative agreement (relative specificity), 100 sera from blood donors were analyzed.

| | negative agreement (relative specificity) | | positive agreement (relative sensitivity) | |
|-------------|--|----------|--|----------|
| | in % | absolute | in % | absolute |
| nukleosomes | 100 % | 100/100 | 100 % | 10/10 |
| dsDNA | 100 % | 100/100 | 100 % | 12/12 |
| histones | 100 % | 100/100 | 94 % | 17/18 |
| SmD1 | 100 % | 100/100 | 100 % | 15/15 |
| U1-snRNP | 98 % | 98/100 | 100 % | 16/16 |
| SS-A/Ro60kD | 100 % | 100/100 | 100 % | 28/28 |
| SS-A/Ro52kD | 100 % | 100/100 | 100 % | 23/23 |
| SS-B/La | 100 % | 100/100 | 100 % | 14/14 |
| Scl-70 | 100 % | 100/100 | 100 % | 12/12 |
| CENP-B | 100 % | 100/100 | 100 % | 20/20 |
| Jo-1 | 100 % | 100/100 | 100 % | 10/10 |
| Rib-P0 | 100 % | 100/100 | 100 % | 7/7 |

11 Literature

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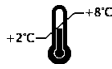

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For further reading:

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Tan EM, (1989). Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol* 44: 93–151.

| | | |
|--|--|-------------------------------------|
| IVD | " Diagnosi in vitro | " For in vitro diagnostic use |
| | " Pour diagnostic in vitro | " Para uso diagnóstico in vitro |
| | " In Vitro Diagnostikum | " In Vitro Διαγνωστικό μέσο |
| | " Para uso Diagnóstico in vitro | |
| REF | " Numero d'ordine | " Catalogue number |
| | " Référence Catalogue | " Numéro de catálogo |
| | " Bestellnummer | " Αριθμός παραγγελίας |
| | " Número de catálogo | |
| LOT | " Descrizione lotto | " Lot |
| | " Lot | " Lote |
| | " Chargen Bezeichnung | " Χαρακτηρισμός παρτίδας |
| | " Lote | |
| CE | " Conformità europea | " EC Declaration of Conformity |
| | " Déclaration CE de Conformité | " Declaración CE de Conformidad |
| | " Europäische Konformität | " Ευρωπαϊκή συμφωνία |
| | " Declaração CE de Conformidade | |
|  | " 24 determinazioni | " 24 tests |
| | " 24 tests | " 24 pruebas |
| | " 24 Bestimmungen | " 24 προσδιορισμοί |
| | " 24 Testes | |
|  | " Rispettare le istruzioni per l'uso | " See instructions for use |
| | " Voir les instructions d'utilisation | " Ver las instrucciones de uso |
| | " Gebrauchsanweisung beachten | " Λάβετε υπόψη τις οδηγίες χρήσης |
| | " Ver as instruções de uso | |
|  | " Da utilizzarsi entro | " Use by |
| | " Utilise avant le | " Utilizar antes de |
| | " Verwendbar bis | " Χρήση μέχρι |
| | " Utilizar antes de | |
|  | " Conservare a 2-8 °C | " Store at 2-8 °C (35-46 °F) |
| | " Conserver à 2-8 °C | " Conservar a 2-8 °C |
| | " Lagerung bei 2-8 °C | " Φυλάσσεται στους 2-8 °C |
| | " Conservar entre 2-8 °C | |
|  | " Prodotto da | " Manufactured by |
| | " Fabriqué par | " Fabricado por |
| | " Hergestellt von | " Κατασκευάζεται από |
| | " Fabricado por | |
| STRIP | " Strip di nitrocellulosa rivestita | " Coated nitrocellulose strip |
| | " Strip de nitrocellulose couché | " Tira de nitrocellulosa recubierta |
| | " Nitrozellulosemembran-Streifen mit aufgebracht Antigenen | " Επίστρωση λωρίδα νιτροκυταρίνης |
| | " Tira de nitrocellulose revestido | |
| WASH 20x | " Tamponi di lavaggio | " Wash buffer |
| | " Tampon de Lavage | " Solución de lavado |
| | " Waschpuffer | " Ρυθμιστικό διάλυμα πλύσης |
| | " Solução de lavagem | |
| Block-Reag | " Reagente bloccante | " Blocking Reagent |
| | " réactif de blocage | " Reactivo bloqueante |
| | " Blockier-Reagenz | " Αντιδραστήριο αποκλεισμού |
| | " Bloqueio de reagente | |
| RCNS 10ml | " Ricostituire con 10 mL | " Reconstitute with 10 mL |
| | " reconstituer avec 10 mL | " reconstituir con 10 mL |
| | " rekonstituieren mit 10 mL | " Ανασύσταση με 10 mL |
| | " reconstituir com 10 mL | |
| SB | " Tamponi campione | " Sample buffer |
| | " Tampon Echantillons | " Tampón Muestras |
| | " Probenpuffer | " Ρυθμιστικό διάλυμα δειγμάτων |
| | " Diluente de amostra | |
| CONJ | " Coniugato | " Conjugate |
| | " Conjugé | " Conjugado |
| | " Konjugat | " Σύζευγμα |
| | " Conjugado | |
| SUB | " Tamponi substrato | " Substrate buffer |
| | " Substrat | " Tampón sustrato |
| | " Substratpuffer | " Ρυθμιστικό διάλυμα υποστρώματος |
| | " Substrato | |

