



**REF 10104**

**U1-70**





# Instruction Manual

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|   |                 |                 |
|---|-----------------|-----------------|
| <br>autoimmune diagnostic assays | Product Ref.    | 10104           |
|   | Product Desc.   | U1-70           |
|   | Manual Rev. No. | 005: 2024-06-12 |

## 1 Intended Use

**U1-70** is a solid phase enzyme immunoassay employing recombinant human 70 kDa protein of the U1-snRNP complex for the quantitative detection of antibodies against the 70 kDa U1-RNP in human serum.

The assay is a tool in the diagnosis of mixed connective tissue diseases (MCTD) and systemic lupus erythematosus (SLE).

## 2 Clinical Application and Principle of the Assay

The U1-snRNP complex is a small nuclear ribonucleoprotein particle composed of uridine rich (thus U) small nuclear RNA and a set of proteins: the 70 kDa U1-specific ribonucleoprotein plus proteins A and C (all formerly summarized as RNPs) and the Sm (Smith) antigen which comprises eight proteins: B, B', D1, D2, D3, E, F, and G. Because of its protein components, the Sm and RNPs, the complex is often named RNP/Sm complex. The U1-snRNP complex is a part of the splicosomal complex, that facilitates the processing of pre-mRNA to mature mRNA in the nucleus.

Antibodies against the 70 kDa protein of the U1-snRNP complex belong to the heterogenous group of anti-nuclear antibodies (ANA), which are associated with various autoimmune diseases. They are directed against various proteins of the nucleus. 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 but more specific testing by ELISAs employing the target antigens are available too for a simple and reliable differentiation of ANAs.

Antibodies against the 70 kDa U1-specific protein are found in 95% of mixed connective tissue diseases (MCTD) but do also occur in systemic lupus erythematosus (SLE) with a prevalence of 40%. Isolated occurrence of anti-70 kDa antibodies is typical for the Sharp syndrome. Whilst antibodies against Sm are highly specific for systemic lupus erythematosus (SLE) and thus are included in diagnostic and classification criteria for SLE. Anti-Sm antibodies are found in 20-30% of patients with SLE.

### Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. 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 in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

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### 3 Kit Contents

| TO BE RECONSTITUTED   |                    |           |                |  |
|---|--------------------|-----------|----------------|--|
| Item  | Quantity           | Cap color | Solution color | Description / Contents   |
| Sample Buffer (5x)  | 1 x 20ml           | White     | Yellow         | 5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)  |
| Wash Buffer (50x)   | 1 X 20ml           | White     | Green          | 50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)   |
| READY TO USE  |                    |           |                |  |
| Item  | Quantity           | Cap color | Solution color | Description / Contents   |
| Negative Control  | 1 x 1.5ml          | Green     | Colorless      | Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)  |
| Positive Control  | 1 x 1.5ml          | Red       | Yellow         | Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)  |
| Calibrators   | 6 x 1.5ml          | White     | Yellow *       | Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative) |
| Conjugate, IgG  | 1 x 15ml           | Blue      | Blue           | Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA),   |
| TMB Substrate   | 1 x 15ml           | Black     | Colorless      | Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H <sub>2</sub> O <sub>2</sub> )   |
| Stop Solution   | 1 x 15ml           | White     | Colorless      | 1M Hydrochloric Acid   |
| Microtiter plate  | 12 x 8 well strips | N/A       | N/A            | With breakaway microwells. Refer to paragraph 1 for coating.   |
| * Color increasing with concentration   |                    |           |                |  |
| MATERIALS REQUIRED, BUT NOT PROVIDED  |                    |           |                |  |
| Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent 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 the microplate at 2-8°C/35.6-46.4°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35.6-46.4°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

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## 5 Precautions of Use

### 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 in conditions of the 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 to avoid contact with eyes and skin and wear disposable gloves.

**WARNING !** Calibrators, Controls and Buffers contain sodium azide ( $\text{NaN}_3$ ) as a preservative.  $\text{NaN}_3$  may be toxic if ingested or adsorbed by skin or eyes.  $\text{NaN}_3$  may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

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

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

### 5.2 General directions for use

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

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

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.

**Incubation: We recommend test performance at 30°C/86°F for automated systems.**

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 used with other reagents prior.

**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.**

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## 6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum 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 samples should be used during the first 8h, respectively stored tightly closed at 2-8°C/35.6-46.4°F up to 48h, or frozen at -20°C/-4°F for longer periods

## 7 Assay Procedure

### 7.1 Preparations prior to starting

#### Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

#### Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

#### Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

#### Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

#### Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

#### Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35.6-46.4°F).

## 7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

|   | 1     | 2     | 3   | 4... |
|---|-------|-------|-----|------|
| A | Cal A | Cal E | P1  |      |
| B | Cal A | Cal E | P1  |      |
| C | Cal B | Cal F | P2  |      |
| D | Cal B | Cal F | P2  |      |
| E | Cal C | PC    | P3  |      |
| F | Cal C | PC    | P3  |      |
| G | Cal D | NC    | ... |      |
| H | Cal D | NC    | ... |      |

CalA: calibrator A

CalB: calibrator B

CalC: calibrator C

CalD: calibrator D

CalE: calibrator E

CalF: calibrator F

PC: positive control



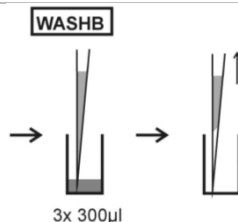
NC: negative control

P1: patient 1


P2: patient 2



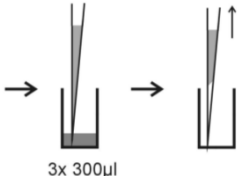
P3: patient 3

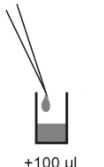

## 7.3 Test Steps

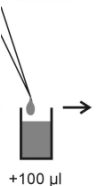

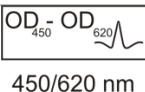
| Step                          | Description  |
|-------------------------------|--|
| 1.                            | Ensure preparations from step 7.1 above have been carried out prior to pipetting.  |
| 2.                            | Use the following steps in accordance with quantitative interpretation results desired:  |
| <b>CONTROLS &amp; SAMPLES</b> |  |
| 3.                            |  <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:<br/>Calibrators (CAL.A to CAL.F)<br/>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> <li>Negative control (NC) and Positive control (PC), and</li> <li>Patients diluted serum (P1, P2...)</li> </ul> |
| 4.                            |  <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>   |
| 5.                            |  <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>  |



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| CONJUGATE |   |  |
|-----------|---|--|
| 6.        | <div><div>CONJ</div><div></div></div>  | Pipette 100 µl conjugate into each well.           |
| 7.        | <div></div>                            | Incubate for 30 minutes at 20-32°C/68-89.6°F.      |
| 8.        | <div><div>WASHB</div><div></div></div> | Wash 3x with 300 µl washing buffer (diluted 1:50). |

| SUBSTRATE |  |   |
|-----------|--|---|
| 9.        | <div><div>SUB</div><div></div></div> | Pipette 100 µl TMB substrate into each well.                                |
| 10.       | <div><div></div></div>              | Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light. |

| STOP |  |   |
|------|--|---|
| 11.  | <div><div>STOP</div><div></div></div>   | Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate. |
| 12.  | <div><div></div></div>  | Incubate 5 minutes minimum.   |
| 13.  |  | Agitate plate carefully for 5 sec.  |
| 14.  | <div><div><div>OD<sub>450</sub> - OD<sub>620</sub></div><div></div><div>450/620 nm</div></div></div> <td>Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.</td> | Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.                         |

## 8 Quantitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

| Normal Range | Equivocal Range | Positive Results |
|--------------|-----------------|------------------|
| < 12 U/ml    | 12 - 18 U/ml    | >18 U/ml         |

### *Example of a standard curve*

**Do NOT use this example for interpreting patient's result**

| Calibrators IgG | OD 450/620 nm | CV % (Variation) |
|-----------------|---------------|------------------|
| 0 U/ml          | 0.044         | 3.2              |
| 3 U/ml          | 0.132         | 0.6              |
| 10 U/ml         | 0.300         | 2.1              |
| 30 U/ml         | 0.557         | 2.8              |
| 100 U/ml        | 1.224         | 1.9              |
| 300 U/ml        | 2.130         | 0.3              |

### *Example of calculation*

| Patient | Replicate (OD) | Mean (OD) | Result (U/ml) |
|---------|----------------|-----------|---------------|
| P 01    | 1.156/1.108    | 1.132     | 86.9          |
| P 02    | 0.543/0.564    | 0.554     | 29.9          |

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

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## 9 Technical Data

|                           |   |
|---------------------------|---|
| Sample material:          | serum   |
| Sample volume:            | 10 µl of sample diluted 1:101 with 1x sample buffer |
| Total incubation time:    | 90 minutes at 20-32°C/68-89.6°F                     |
| Calibration range:        | 0-300 U/ml  |
| Analytical sensitivity:   | 0.83 U/ml   |
| Storage:                  | at 2-8°C/35.6-46.4°F use original vials only.       |
| Number of determinations: | 96 tests  |

## 10 Performance Data

### 10.1 Normal Range

Sera of healthy donors have been investigated on U1-70 and resulted in the following distribution:

| Number of Samples | negative   | borderline | positive |
|-------------------|------------|------------|----------|
| 80                | 80 (100 %) | 0 (0 %)    | 0 (0%)   |

We also recommend that each laboratory should establish its own normal range.

### 10.2 Precision

Precision of test results obtained with U1-70, REF 10104 were assessed by the determination of the intra- and inter assay precision as well as the lot-to-lot variance by the analysis of multiple samples of different antibody activities.

| Sample ID | Intra Assay Precision |      | Inter Assay Precision |       | LOT to LOT Precision |      |
|-----------|-----------------------|------|-----------------------|-------|----------------------|------|
|           | Mean (U/ml)           | CV   | Mean (U/ml)           | CV    | Mean (U/ml)          | CV   |
| Sample 1  | 8.38                  | 8.4% | 8.38                  | 11.0% | 8.65                 | 8.5% |
| Sample 2  | 18.58                 | 5.7% | 18.58                 | 7.3%  | 19.13                | 7.0% |
| Sample 3  | 31.82                 | 5.7% | 31.82                 | 9.7%  | 33.78                | 4.1% |
| Sample 4  | 65.94                 | 6.3% | 65.94                 | 12.9% | 71.84                | 5.9% |
| Sample 5  | 205.91                | 5.4% | 205.91                | 7.6%  | 214.21               | 4.4% |

### 10.3 Sensitivity and Specificity

#### Analytical sensitivity

The analytical sensitivity has been assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection.  
For U1-70, REF 10104 a **LoD of 0.83 U/ml** has been determined.

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## 10.4 Linearity

Three sera covering the whole test range were diluted serially with a negative serum sample. Measured and expected values of the distinct dilutions were used to calculate a linear regression. According to results of linearity testing a measurable range of 3 - 300 U/ml was determined for U1-70.

## 10.5 Calibration

The U1-70 is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta. The results are expressed in U/ml.

## 11 Disposal

Please observe the relevant statutory requirements!

## 12 Literature

**Peter JB, Shoenfeld Y (1996).** Autoantibodies. Elsevier Sciences B.V., Amsterdam

**Hackl W, Fischer U, Luhrmann R (1994).** A 69 kD protein that associates reversibly with the Sm core domain of several spliceosomal snRNP species. J Cell Biol 124: 261-272.






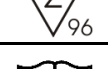

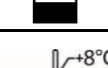











**Guldner HH (1992)** Mapping of epitopes recognized by anti-(U1)RNP antibodies. Mol Biol Rep 16: 155-164.

**Klein Gunnewiek JMT, Van de Putte LBA, van Venrooij WJ (1997).** The U1 snRNP complex: An autoantigen in connective tissue diseases: An update. Clin Exp Rheumatol 15: 549-560.

**Von Mühlen CA, Tan EM (1995).** Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 24: 323-358.

**Lothar Thomas:** Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books

**CLSI Guideline GP44-A4:** Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

|   |   |  |
|---|---|--|
|    | - Diagnosi in vitro<br>- Pour diagnostic in vitro<br>- In Vitro Diagnostikum<br>- Para uso Diagnóstico in vitro   | - For in vitro diagnostic use<br>- Para uso diagnóstico in vitro<br>- In Vitro Διαγνωστικό μέσο          |
|    | * Numero d'ordine<br>* Référence Catalogue<br>* Bestellnummer<br>* Número de catálogo   | * Catalogue number<br>* Numéro de catalogue<br>* Αριθμός παραγγελίας                                     |
|    | * Descrizione lotto<br>* Lot<br>* Chargen Bezeichnung<br>* Lote   | * Lot<br>* Lote<br>* Χαρακτηρισμός παρτίδας  |
|    | * Identificatore univoco del dispositivo<br>* Identifiant unique de l'appareil<br>* eindeutige Produktidentifizierung<br>* Identificador único do dispositivo | * Unique Device Identifier<br>* Identificador único del dispositivo<br>* Μοναδικό αναγνωριστικό συσκευής |
|    | * Conformità europea<br>* Déclaration CE de Conformité<br>* Europäische Konformität<br>* Declaração CE de Conformidade  | * EC Declaration of Conformity<br>* Declaración CE de Conformidad<br>* Ευρωπαϊκή συμφωνία                |
|    | * 96 determinazioni<br>* 96 tests<br>* 96 Bestimmungen<br>* 96 Testes   | * 96 tests<br>* 96 pruebas<br>* 96 προσδιορισμοί   |
|    | * Rispettare le istruzioni per l'uso<br>* Voir les instructions d'utilisation<br>* Gebrauchsanweisung beachten<br>* Ver as instruções de uso                  | * See instructions for use<br>* Ver las instrucciones de uso<br>* Λάβετε υπόψη τις οδηγίες χρήσης        |
|    | * Da utilizzarsi entro<br>* Utiliser avant le<br>* Verwendbar bis<br>* Utilizar antes de  | * Use by<br>* Utilizar antes de<br>* Χρήση μέχρι   |
|   | * Conservare a 2-8°C<br>* Conserver à 2-8°C<br>* Lagerung bei 2-8°C<br>* Conservar entre 2-8°C  | * Store at 2-8°C (35.6-46.4°F)<br>* Conservar a 2-8°C<br>* Φυλάσσεται στους 2-8°C                        |
|  | * Prodotto da<br>* Fabriqué par<br>* Hergestellt von<br>* Fabricado por   | * Manufactured by<br>* Fabricado por<br>* Κατασκευάζεται από   |
|  | * Controllo positivo<br>* Contrôle Positif<br>* Positiv Kontrolle<br>* Controllo positivo   | * Positive Control<br>* Control Positivo<br>* Θετικός ορός ελέγχου                                       |
|  | * Controllo negativo<br>* Contrôle Négatif<br>* Negativ Kontrolle<br>* Controllo negativo   | * Negative Control<br>* Control Negativo<br>* Αρνητικός ορός ελέγχου                                     |
|  | * Calibratore<br>* Etalon<br>* Kalibrator<br>* Calibrador   | * Calibrator<br>* Calibrador<br>* Αντιδραστήριο βαθμονόμησης   |
|  | * Coniugato<br>* Conjugé<br>* Konjugat<br>* Conjugado   | * Conjugate<br>* Conjugado<br>* Σύζευγμα   |
|  | * Micropiastra rivestita<br>* Microplaque sensibilisée<br>* Beschichtete Mikrotiterplatte<br>* Microplaca revestida   | * Coated microtiter plate<br>* Microplaca sensibilizada<br>* Επικαλυμμένη μικροτρίδακα                   |
|  | * Tampone di lavaggio<br>* Tampon de Lavage<br>* Waschpuffer<br>* Solução de lavagem  | * Wash buffer<br>* Solución de lavado<br>* Ρυθμιστικό διάλυμα πλύσης                                     |
|  | * Tampone substrato<br>* Substrat<br>* Substratpuffer<br>* Substrato  | * Substrate buffer<br>* Tampón sustrato<br>* Ρυθμιστικό διάλυμα υποστρώματος                             |
|  | * Reagente bloccante<br>* Solution d'Arrêt<br>* Stopreagenz<br>* Solução de paragem   | * Stop solution<br>* Solución de parada<br>* Αντιδραστήριο διακοπής αντίδρασης                           |
|  | * Tampone campione<br>* Tampon Echantillons<br>* Probenpuffer<br>* Diluente de amostra  | * Sample buffer<br>* Tampón Muestras<br>* Ρυθμιστικό διάλυμα δειγμάτων                                   |