



REF 10150
Histone-C



Instruction Manual

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| | | |
|---|-----------------|------------------|
|  | Product Ref. | 10150 |
| | Product Desc. | Histone-C |
| | Manual Rev. No. | 005 : 2014-01-15 |

1 Intended Use

Histone-C is a solid phase enzyme immunoassay with human native histones H1, H2A, H2B, H3, H4 isolated from the eukaryotic celline HeLa for the quantitative detection of antibodies against histones in human serum.

The assay is a tool in the differential diagnosis of systemic lupus erythematosus (SLE).

2 Clinical Application and Principle of the Assay

Histones are small basic proteins of 11 to 21 kDa constituting a set of interacting proteins that organize and constrain the topology of DNA in most eukaryotic cells into a particle called nucleosome. In this particle the four histones H2A, H2B, H3 and H4 form an octamer (H2A, H2B, H3, H4)₂ around which 146 bp of dsDNA is wrapped by 1 ¾ turns. Histone H1 interacts with the nucleosome and together with linked-DNA connects neighboring nucleosomes.

Antibodies against histones occur in patients with SLE with a frequency of 50 %, during active phases of SLE with an even higher frequency (80%).

They are not specific for SLE, especially when not associated with anti-dsDNA antibodies. They are also found in other inflammatory rheumatoid diseases, like rheumatoid arthritis (low titers and mainly against H1), or systemic sclerosis and juvenile chronic arthritis. Moreover these antibodies are found in primary biliary cirrhosis (up to 70%) and autoimmune hepatitis (up to 35%), too .

Anti-histone antibodies, in the absence of antibodies to dsDNA, do play an important role in the diagnosis of drug-induced LE being found in 95% of cases. These drugs are inducing joint and skin symptoms, as well as the different anti-histone specificities, while never renal manifestations. Anti-histone antibodies in drug-induced LE are temporary and disappear within a few months after withdrawal of the inducing drug.

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.

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 ₂ O ₂) |
| 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/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for at least 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 (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ 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 Controls, Calibrators, Conjugates 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/35-46°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/35-46°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



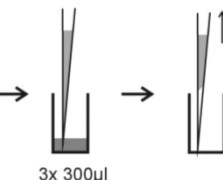
NC: negative control


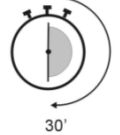
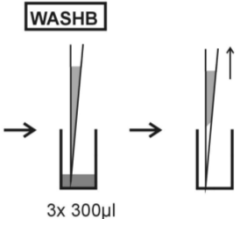

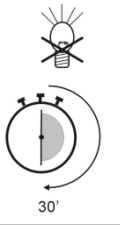
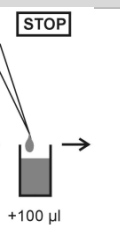

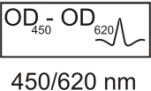
P1: patient 1

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: |
| CONTROLS & SAMPLES | |
| 3. | <div style="display: flex; align-items: center;">  <div style="margin-left: 20px;"> <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:</p> <p style="margin-left: 20px;">Calibrators (CAL.A to CAL.F)</p> <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> • Negative control (NC) and Positive control (PC), and • Patients diluted serum (P1, P2...) </div> </div> |
| 4. | <div style="display: flex; align-items: center;">  <div style="margin-left: 20px;"> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p> </div> </div> |
| 5. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;"> <p>WASHB</p>  </div> <div> <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p> </div> </div> |

| CONJUGATE | |
|-----------|---|
| 6. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl conjugate into each well.</p> </div> </div> |
| 7. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p> </div> </div> |
| 8. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p> </div> </div> |
| SUBSTRATE | |
| 9. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl TMB substrate into each well.</p> </div> </div> |
| 10. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.</p> </div> </div> |
| STOP | |
| 11. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.</p> </div> </div> |
| 12. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate 5 minutes minimum.</p> </div> </div> |
| 13. | <p>Agitate plate carefully for 5 sec.</p> |
| 14. | <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.</p> </div> </div> |

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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 | 0.028 | 3.1 |
| 3 U/ml | 0.132 | 1.6 |
| 10 U/ml | 0.281 | 1.3 |
| 30 U/ml | 0.642 | 1.4 |
| 100 U/ml | 1.391 | 0.3 |
| 300 U/ml | 2.389 | 0.1 |

Example of calculation

| Patient | Replicate (OD) | Mean (OD) | Result (U/ml) |
|---------|----------------|-----------|---------------|
| P 01 | 1.402/1.420 | 1.411 | 103.5 |
| P 02 | 0.637/0.615 | 0.627 | 29.2 |

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: | 1.0 U/ml |
| Storage: | at 2-8°C/35-46°F use original vials only. |
| Number of determinations: | 96 tests |

10 Performance Data

10.1 Analytical sensitivity

Testing sample buffer 30 times on Histone-C gave an analytical sensitivity of 1.0 U/m

10.2 Specificity and sensitivity

The microplate is coated with native human Histones H1, H2A, H2B, H3, H4. No crossreactivities to other autoantigens have been found. Antibodies against histones occur in 50% of SLE patients, during active phases of SLE with an even higher frequency (80%).

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

| Sample No. | Dilution Factor | Measured (U/ml) | Expected (U/ml) | Recovery (%) |
|------------|-----------------|-----------------|-----------------|--------------|
| 1 | 1 / 100 | 82.1 | 86.2 | 95.2 |
| | 1 / 200 | 40.2 | 43.1 | 93.3 |
| | 1 / 400 | 23.3 | 21.6 | 107.9 |
| | 1 / 800 | 11.3 | 10.8 | 104.6 |
| 2 | 1 / 100 | 144.0 | 142.0 | 101.4 |
| | 1 / 200 | 75.8 | 71.0 | 106.8 |
| | 1 / 400 | 37.7 | 35.5 | 106.2 |
| | 1 / 800 | 16.2 | 17.8 | 91.0 |

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10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

| Intra-assay | | |
|-------------|-------------|--------|
| Sample No. | Mean (U/ml) | CV (%) |
| 1 | 102.2 | 6.7 |
| 2 | 44.9 | 3.7 |
| 3 | 4.4 | 0.4 |

| Inter-assay | | |
|-------------|-------------|--------|
| Sample No. | Mean (U/ml) | CV (%) |
| 1 | 208.2 | 8.7 |
| 2 | 155.6 | 7.9 |
| 3 | 45.2 | 7.6 |

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11 Literature








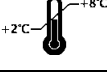

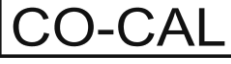










Gioud M, Ait Kaci A, Monier JC (1982). Histone antibodies in systemic lupus erythematosus. A possible diagnostic tool. *Arthritis Rheum* 25: 407-413.

Rubin RL, Joslin FG, Tan EM (1982). Specificity of anti-histone antibodies in systemic lupus erythematosus *Arthritis Rheum* 25: 779-782.

Shoenfeld Y and Segol O (1989). Anti-histone antibodies in SLE and other autoimmune diseases. *Clin and Exp Rheum* 7: 265-271.

Cohen MG and Welb J (1989). Anti-histone antibodies in rheumatoid arthritis and Felty's syndrome. *Arthr. Rheum* 32: 10.

Burlingame RW (1997). The clinical utility of anti-histone antibodies. *Clinics in Laboratory Medicine* 17: 367-378.

| | | |
|---|---------------------------------------|---|
|  | - 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 | |
|  | ° Numero d'ordine | ° Catalogue number |
| | ° Référence Catalogue | ° Numéro de catálogo |
| | ° Bestellnummer | ° Αριθμός παραγγελίας |
|  | ° Número de catálogo | |
| | ° Descrizione lotto | ° Lot |
| | ° Lot | ° Lote |
| | ° Chargen Bezeichnung | ° Χαρακτηρισμός παρτίδας |
|  | ° Lote | |
| | ° Conformità europea | ° EC Declaration of Conformity |
| | ° Déclaration CE de Conformité | ° Declaración CE de Conformidad |
| | ° Europäische Konformität | ° Ευρωπαϊκή συμφωνία |
|  | ° Déclaracão CE de Conformidade | |
| | ° 96 determinazioni | ° 96 tests |
| | ° 96 tests | ° 96 pruebas |
| | ° 96 Bestimmungen | ° 96 προσδιορισμοί |
|  | ° 96 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 utilizarsi 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 | |
| | ° Calibratore cut-off | ° Cut off Calibrator |
| | ° Etalon Seuil | ° Calibrador de cut-off |
| | ° Grenzwert Kalibrator | ° Οριακός ορός Αντιδραστήριο βαθμονόμησης |
|  | ° Calibrador de cut-off | |
| | ° Controllo positivo | ° Positive Control |
| | ° Contrôle Positif | ° Control Positivo |
| | ° Positiv Kontrolle | ° Θετικός ορός ελέγχου |
|  | ° Controllo positivo | |
| | ° Controllo negativo | ° Negative Control |
| | ° Contrôle Négatif | ° Control Negativo |
| | ° Negativ Kontrolle | ° Αρνητικός ορός ελέγχου |
|  | ° Controllo negativo | |
| | ° Calibratore | ° Calibrator |
| | ° Etalon | ° Calibrador |
| | ° Kalibrator | ° Αντιδραστήριο βαθμονόμησης |
|  | ° Calibrador | |
| | ° Recupero | ° Recovery |
| | ° Corrélation | ° Recuperado |
| | ° Wiederfindung | ° Ανάκτηση |
|  | ° Recuperação | |
| | ° Coniugato | ° Conjugate |
| | ° Conjugé | ° Conjugado |
| | ° Konjugat | ° Σύζευγμα |
|  | ° Conjugado | |
| | ° Micropiastra rivestita | ° Coated microtiter plate |
| | ° Microplaque sensibilisée | ° Microplaca sensibilizada |
| | ° Beschichtete Mikrotiterplatte | ° Επικαλυμμένη μικροπλάκα |
|  | ° Microplaca revestida | |
| | ° Tampone di lavaggio | ° Wash buffer |
| | ° Tampon de Lavage | ° Solución de lavado |
| | ° Waschpuffer | ° Ρυθμιστικό διάλυμα πλύσης |
|  | ° Solução de lavagem | |
| | ° Tampone substrato | ° Substrate buffer |
| | ° Substrat | ° Tampón sustrato |
| | ° Substratpuffer | ° Ρυθμιστικό διάλυμα υποστρώματος |
|  | ° Substrato | |
| | ° Reagente bloccante | ° Stop solution |
| | ° Solution d'Arrêt | ° Solución de parada |
| | ° Stopreagenz | ° Αντιδραστήριο διακοπής αντίδρασης |
|  | ° Solução de paragem | |
| | ° Tampone campione | ° Sample buffer |
| | ° Tampon Echantillons | ° Tampón Muestras |
| | ° Probenpuffer | ° Ρυθμιστικό διάλυμα δειγμάτων |
| | ° Diluente de amostra | |