Creative Diagnostics®

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User's Manual

Anti-Hepatitis A-Virus (HAV) IgM ELISA Kit

REF **DEIA-NB24-07M**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Anti-HAV IgM ELISA Kit is an enzyme immunoassay for research use for the qualitative and quantitative detection of IgM antibodies in human serum or plasma directed against the Hepatitis A-Virus.

General Description

The detection of anti-HAV IgM (IgM antibodies specific for Hepatitis A virus) in the serum or plasma of patients indicates a fresh infection with the Hepatitis-A virus. Depending on the anti HAV IgM titre it is possible to differentiate between an acute infection (1 - 3 months after the start of clinical symptoms) and early convalescence (3 - 6 months). In rare cases (approx. 10% of all clinical cases) the anti-HAV IgM response remains positive (6 - 12 months).

Principles of Testing

The Anti-HAV IgM ELISA Kit is a "class-capture" enzyme immuoassay. Serum or plasma samples are diluted 1:2000 and added to the wells of a microtiter plate, which have been previously coated with an antibody directed against human IgM antibodies (µ-chain specific). All IgM antibodies present in the sample are bound during an incubation step. After washing, the Hepatitis A-virus is added, which binds only to the HAV-specific IgM antibodies. The bound antigen is detected by the addition of the conjugate (monoclonal anti-HAV antibody, peroxidase conjugated). After washing and incubation with a colorimetric substrate the reaction is terminated by addition of stop solution and the color turns to yellow. The absorbance of the coloured reaction product is measured on a microtiter plate reader. The colour intensity of the reaction corresponds to the concentration of antibodies in the sample.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with an antibody directed against human IgM antibodies (anti-µ-chain). Wells are separately breakable. (8x12) wells
2. **Conjugate Concentrate (KK):** 100-fold concentrated (peroxidase labeled Anti-HAV IgG). 1 x 250 µL
3. **Hepatitis A Virus antigen (AG):** inactivated, ready for use. 1 x 13.5 mL
4. **Positive Control (PK):** ready for use, recalcificated human plasma, reactive for anti-HAV-IgM, Titre > 1:10000. 1 x 2 mL
5. **Negative Control (NK):** ready for use, recalcificated human plasma, not reactive for anti-HAV-IgM/IgG. Anti-HBsAg, anti-HIV and anti-HCV negative. 1 x 1 mL
6. **Dilution Buffer (VP):** ready for use. Please shake before use! 1 x 120 mL
7. **Washing Buffer (WP):** 20-fold concentrated solution. 1 x 50 mL
8. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine (TMB). 1 x 12 mL
9. **Stopping Solution (SL):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
10. **Sealing Tape:** for covering the microtiter plate. 2

Materials Required But Not Supplied

1. Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.), 950 mL.
2. Precision pipettes and multichannel pipettes with disposable plastic tips
3. Polyethylene PE/Polypropylene PP tubes for dilution of samples
4. Incubator (suitable for incubations at 37°C)
5. Vortex-mixer
6. Microtiter plate washer (recommended)
7. Microplate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

Storage

Store the kit at 2-8°C after receipt until its expiry date. Avoid repeated thawing and freezing.

The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The 1:20 diluted Washing Buffer WP is 4 weeks stable at 2-8°C

Specimen Collection And Preparation

1. Sample type: Serum and EDTA-plasma

2. Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided.

3. Required sample volume: 10 µL

4. Sample dilution

• Qualitative Test: Samples should be diluted in PE/PP-tubes. For larger sample numbers usage of a multi stepper is recommended. Recommended dilution for Qualitative Test: 1:2000 with Dilution Buffer VP.

• Quantiatitve Test: For quantitative results the samples are diluted 1:2000, 1:20000, 1:200000, 1:2000000 with Dilution Buffer VP.

Example Dilution Protocol

1:20 Pre-Dilution - 190 µL Dilution Buffer VP + 10 µL Serum- or EDTA-Plasma

The pre-diluted sample should then be diluted as follows:

1:2000 Dilution - Add 10 µL of the 1:20 pre-diluted sample to 990 µL Dilution Buffer VP

1:20000 Dilution - Add 100 µL of the 1:2000 dilution to 900 µL Dilution Buffer VP

1:200000 Dilution - Add 100 µL of the 1:20000 dilution to 900 µL Dilution Buffer VP

1:2000000 Dilution - Add 100 µL of the 1:200000 dilution to 900 µL Dilution Buffer VP

After mixing use 100 µL each of the needed sample dilution per well

It is recommended to use the diluted sample within one hour.

Reagent Preparation

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.

1. The required volume of Conjugate Concentrate KK is prepared by 1:100 dilution with Dilution Buffer VP. (e.g. 10 µL KK + 990 µL VP) Prepare only the amount required. The diluted conjugate can be stored for at least one week at 4°C.
2. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest. (e.g. 50 mL WP + 950 mL A.dest) Please dilute only according to daily requirements.

Assay Procedure

Note

1. Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution S, is photosensitive–store and incubation in the dark.
2. When performing the assay reagents and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, diluted Conjugate Concentrate KK, as well as the Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution SL should be added to the plate in the same order as Substrate Solution S. All determinations should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
3. Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems. All washing must be performed with the provided Washing Buffer WP diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

   Automatic washing: When using an Automatic microtiter plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

   Manual washing: Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.
4. The danger of handling with potentially infectious material must be taken into account.

Assay Step

1. Set test samples (1:2000 diluted), Positive Control, Negative Control wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in

duplicate.

2. Prepare Standards: Aliquot 100ul of Positive Control, Negative Control, test samples into wells.
3. Incubate: Seal the plate with a cover and incubate at 37°C for 1 hours.
4. Wash: Aspirate the contents of the wells, and wash plate 3 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
5. Add 100ul HAV-Antigen AG Solution into above wells (standard, control and test samples). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 1 hour.
6. Wash: Aspirate the contents of the wells, and wash plate 3 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
7. Add 100ul of 1:100 diluted Conjugate Concentrate into each well, cover the plate and incubate at 37°C in dark within 30 minutes.
8. Wash: Aspirate the contents of the wells, and wash plate 3 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
9. Add 100ul of Substrate Solution S into each well, cover the plate and incubate at 20-25°C in dark within 30 minutes.
10. Stop: Add 100ul Stopping Solution SL into each well.
11. Measure the absorbance within 30 min at 450 nm, with ≥ 590 nm as reference wavelength.

Quality Control

The average absorbance values of the Positive Controls PK, the Negative Controls NK and the samples are calculated. The Negative Control NK extinction value should not exceed 0.1. The difference between extinctions of the Positive Control PK and Negative Control NK must be at least 0.4, otherwise the test is considered invalid.

Calculation

QUALITATIVE RESULTS

Cut-off calculation-The sum of: 30% of the mean absorbance value of the Positive Control PK + mean absorbance value of the Negative Control NK is used as cut-off. Interpretation of positive: Samples with extinction equal or higher than the cut-off (30% of the average of the positive control + extinction average of the negative control) are regarded as positive. Samples with an extinction of ± 10% of the cut-off value are regarded as border line samples. In this case repeat the test.

Example

Positive Control PK	Extinction
1. value	1.120
2. value	1.205
3. value	1.196
mean value	1.174

Negative Control NK	
1. value	0.021
2. value	0.025
mean value	0.023

The exemplary cut-off: 1.174 × 0.3 + 0.023 = 0.375
Exemplary Sera with extinctions > 0.375 are regarded as positive.
Exemplary Border line samples: 0.375 × 1.1 = 0.412 (+ 10%); 0.375 × 0.9 = 0.337 (-10%)
Extinctions between 0.337 and 0.412

QUANTITATIVE TEST

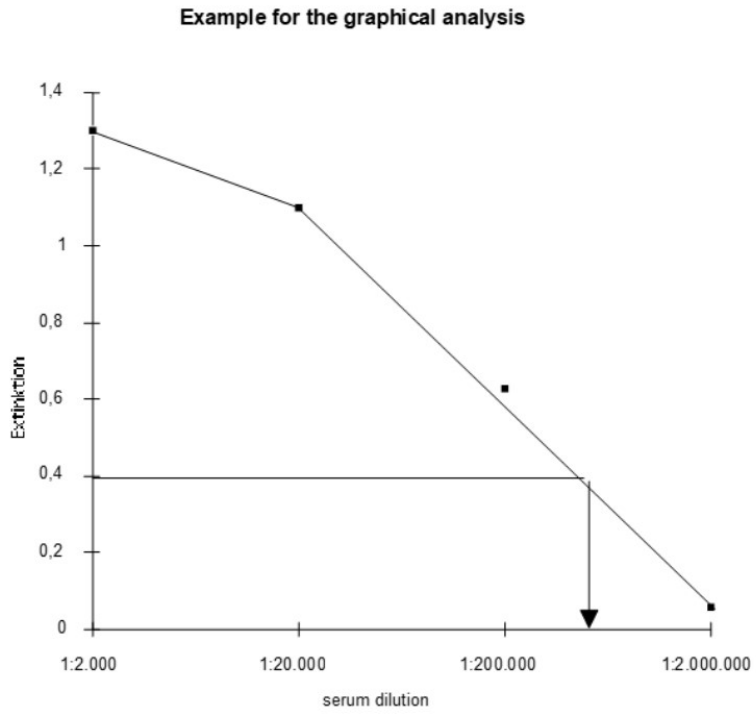
1. Samples preparation: For quantitative results the patients serum is diluted 1:2000, 1:20000, 1: 200000, 1:2000000 with dilution buffer.
2. Test Preparation: Pipette from the above-mentioned serum dilutions, 100 µL per well (we recommend double determinations). The test protocol is identical to the "Qualitative" method.
3. Results: A graph is used to determine the titre in the quantitative calculation of anti-HAV IgM. The graph is plotted on semi-logarithmic paper. The extinction values are plotted on the Y-axis and the serum dilution values on the X-axis.

Cut-off calculation = 30% of the mean absorbance value of the Positive Control PK + mean absorbance value of the Negative Control NK

Example
Positive Control PK average: 1.240
Negative Control NK average: 0.026
cut-off: 1.240 × 0.3 + 0.026 = 0.398

Sample dilution	**Extinction**
1:2000	1.302
1:20000	1.098
1: 200000	0.630
1:2000000	0.060

The measured extinction values of the individual serum dilutions are plotted onto the graph including the cut-off value (drawn parallel to the Y-axis). The individual dots are joined by a line; where this line crosses the limiting value line it is possible to read, from the X-axis, the anti-HAV titre. The serum titre in this example is 1: 500000.



Precautions

1. For research and professional use only. The Creative Diagnostics kit is suitable only for in vitro use and not for internal use in humans and animals.
2. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Creative Diagnostics will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.
3. Do not use obviously damaged or microbial contaminated or spilled material.
4. This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.
5. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.
6. Following components contain human serum: Positive Control, Negative Control, Conjugate Concentrate.

   The antigen has been inactivated with formaldehyde. Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious. The antigen AG has been inactivated with formaldehyde.

Limitations

The influence of the heterophilic antibodies, rheuma factors, anti-species antibodies is reduced by the assay design, but cannot be completely excluded. Higher concentrations of physiological and pharmaceutical or other substances may interfere with the measurement.