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Contents especially technical, methodical and regulations data, may not be amended.

Anti-HAV-IgM ELISA

Enzyme immunoassay for the qualitative and quantitative detection of IgM antibodies against

Hepatitis A-Virus
Product Code: E11

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com



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Gesellschaft für Forschung und Herstellung von Diagnostika GmbH

Aspenhastr. 25 • D-72770 Reutlingen / Germany
Phone: + 49 - (0) 7121 51484-0 • Fax: + 49 - (0) 7121 51484-10
E-Mail: contact@mediagnost.de • <http://www.mediagnost.de>

Read entire protocol before use!

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INTENDED USE

The [mediagnost anti-HAV IgM EIA](#) E11, is an enzyme immunoassay **for research use** and detects IgM antibodies in human serum or plasma directed against the Hepatitis A-Virus.

TEST PRINCIPLE

The mediagnost anti-HAV IgM EIA is a "class-capture" enzyme immunoassay. 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). Specific antibodies in the sample bind to the antibodies present during an incubation step of 1h at 37°C. After washing, the Hepatitis A-virus is added. During an incubation step of 1h at 37°C the HAV binds to the HAV specific IgM antibodies. The conjugate (monoclonal anti-HAV antibody, peroxidase-labelled) is added and incubated again (for 1 h at 37°C). After a further washing step substrate is added and further incubated for 30 min. at room temperature. The reaction is terminated on addition of stop solution. 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.

TEST KIT CONTENT

1) Testplate:

A 96 well test plate, subdivided into 12 strips with 8 wells/each coated with an antibody directed against human IgM antibodies (anti- μ -chain).

2) Conjugate concentrate (KK)

1 vial (250 μ l) conjugate: Peroxidase conjugated anti-HAV-IgG (monoclonal, mouse), 100-fold concentrated.

3) HAV-antigen (AG)

1 vial (13,5 ml) Hepatitis A-Virus antigen, inactivated, ready for use.

4) Positive control (PK)

1 vial (2 ml) positive control. Recalcificated human plasma, reactive for anti-HAV-IgM, Titre > 1:10.000, ready for use.

5) Negative control (NK)

1 vial (1 ml) negative control serum. Human plasma, not reactive for anti-HAV-IgM/IgG. Anti-HBsAg, anti-HIV and anti HCV negative, ready for use.

6) Dilution buffer (VP)

1 vial (120 ml) phosphate buffered saline with stabilizers, for the dilution of serum samples and conjugate, ready for use.

7) Substrate (S)

1 vial (12 ml) substrate (TMB). Ready for use.

8) Stop solution (ST)

1 vial (12 ml) stop solution, ready for use, contains 0.2 M sulphuric acid. **Caution: acid.**

9) Wash buffer, 20 times concentrated (WP)

1 vial (50 ml) wash buffer (phosphate-buffered saline with detergent). 20 times concentrated.

10) Sealing tape for covering of the microtiter plate, 2 x, adhesive.

Storage condition

All test kit materials must be stored between 2 - 8°C in the dark. Strips not to be used immediately should be stored airtight in the laminate bag together with the desiccant. The shelf-life of components after opening is not affected, if used appropriately.

Additional reagents and equipment

- Distillated or demineralised water for the buffer-dilution
- Incubator
- Microplate washer or device capable to dispense and aspirate 300 µl of washing buffer per well
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥ 590 nm
- Precision pipettes with disposable tips
- Polyethylen PE/Polypropylen PP tubes for dilution of samples

PRECAUTIONS

1. The mediagnost anti-HAV IgM EIA E11 is for in-vitro use only!
2. The antigen has been inactivated with formaldehyde. The reagents of human origin have been tested for HBsAg and antibodies to HIV and HCV and found to be negative. Nevertheless, such tests are unable to prove the complete absence of infectious agents. Therefore, all reagents should be handled with appropriate precautions.
3. Do not pipette by mouth. Wear disposable gloves throughout the test procedure. In case of spills, bench-tops and instruments must be disinfected.

4. Disposable materials should be treated as infectious waste.
5. The stop solution contains sulphuric acid and is therefore corrosive. By contact wash immediately with running water - if necessary contact a doctor.
6. Acidic waste should be neutralized before disposal.
7. Reagents contain as preservatives Proclin und Kathon, however, highly diluted (0.01%).

First aid procedures:

Scin contact: Wash affected area thoroughly with water.
Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician. The Stop Solution provided is an acid solution. Avoid direct contact. Wear eye, hand, face and clothing protection when using this material.

TECHNICAL NOTES

Incubation at room temperature means: 20-25°C

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 diluted to usage concentration. Washing volume per washing cycle and well must be 300 µl at least.

The danger of handling with potentially infectious material must be taken into account.

When using an automatic microtitre plate washer, the respective instructions for 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 is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically swinging out the microtitre 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.

QUALITATIVE TEST PROTOCOL

Test Preparation qualitative

1. Bring all reagents to room temperature (20 - 25°C) before use.
2. Dilute the 20 x concentrated washing buffer 1:20 with distilled or deionised water. Attention: After dilution the washing buffer is only 4 weeks stable, please dilute only according to requirements.

3. Remove exactly the number of strips required per test reaction. Unused test strips should be stored together with dessicant in the same Aluminium bag provided.

4. Dilute the conjugate 1:100 with dilution buffer.

Sample Preparation

The mediagnost anti-HAV-IgM EIA can be performed with either human serum or recalcificated plasma. Dilute samples 1:2000 with dilution buffer. Negative and positive control sera are ready for use.

Test procedure

1. For each assay the blank (A1), 3 positive and 2 negative controls should be included. The first well (A1, blank) should be left empty. Add 100 µl of positive (B1, C1 and D1) or negative (E1 and F1) control serum per well.

Dispense 100 µl of samples (duplicates) into wells. The wells of the reaction chambers are sealed with adhesive tape and incubated for 1 hour at 37 °C.

2. The serum is then aspirated and the wells are washed **3 times** with **300 µl Washing buffer** (20 seconds per cycle).

3. Add 100 µl (with the exception of A1, blank) HAV-antigen solution to each well. Then the reaction chambers are sealed with tape and incubated for further 1 hour at 37°C.

4. The wells are then aspirated and washed **3 times** with **300 µl Washing buffer WP** / well. (20 seconds per cycle).

5. Add 100 µl conjugate solution (anti-HAV-IgG, Peroxidase conjugated) to each well (with the exception of A1, blank). The reaction chambers are sealed with tape and incubated for 1 hour at 37°C.

6. The conjugate is aspirated and the wells washed **3 times** with **300 µl Washing buffer WP** / well (20 seconds per cycle).

7. Add 100 ul of substrate solution (TMB) into each well (include A1) and incubate 30 minutes at room temperature **in the dark**.

8. On completion of incubation add 100 µl stop solution into each well (include A1) **Caution: ACID**.

9. Measure the colour reaction within 30 minutes at 450 nm (reference filter ≥ 590 nm).

The absorbance of the blank wells are subsequently subtracted from the control and sera to obtain the absolute values

Qualitative Results

The average of the three positive, the two negative control values and the samples are calculated.

The negative control serum value should not exceed an extinction of 0.1 **and** the difference between the positive and negative control value must be at least 0.400 otherwise the test is considered invalid.

Cut-off calculation:

30% of the positive control average + average of the negative control is used as cut off.

Interpretation

Positive test result:

Samples with an 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.

Border line samples:

Samples with an extinction of $\pm 10\%$ of the cut off value are regarded as border line samples. In this case repeat the test.

Calculation (example)

<u>Positive control</u>	<u>Extinction:</u>
1. value	1.120
2. value	1.205
3. value	1.196
<u>total:</u>	<u>3.521</u>

mean value: $3.521 / 3 = 1,174$

Negative control:

1. value	0,021
2. value	0,025
<u>total:</u>	<u>0,046</u>

mean value: $0,046 / 2 = 0,023$

cut off: $1.174 \times 0,3 + 0,023 = 0,352 + 0,023 = \mathbf{0,375}$

Sera with extinctions $> 0,375$ are regarded as positive.

Border line samples:

$0,375 \times 1,1 = 0,412$ (+ 10%)

$0,375 \times 0,9 = 0,337$ (-10%)

with extinctions between **0,337 and 0,412**

QUANTITATIVE TEST PROTOCOL

To determine the serum titer for example to control the course of disease the quantitative protocol is recommended.

Samples preparation

For quantitative results the sample is diluted 1:2000, 1:20.000, 1: 200.000, 1:2.000.000 with dilution buffer to give end concentrations of: 2×10^{-3} , 2×10^{-4} , 2×10^{-5} , 2×10^{-6} .

Test Preparation

The "blank", the positive and negative serum controls are also included as in the "Qualitative" method. The test protocol is identical to the "Qualitative" method.

Quantitative 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 positive control average + average of the negative control is used as the cut off value.

Titer calculation (example)

Positive control average: 1,240

Negative control average: 0,026

cut-off: $1,240 \times 0,3 + 0,026 = 0,398$

Sample dilution	Extinction
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1:2000	1,302
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1:20 000	1,098
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1: 200 000	0,630
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1:2 000 000	0,060
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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: 500.000.

Example for the graphical analysis

