AccuDiag™ HAV IgM ELISA Kit

REF 1718-P1

IVD See External Label 2-8°C 96 Tests

HAV IgM ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	50 µL serum/plasma
Incubation Time	85 minutes
Sensitivity	100%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

HAV IgM ELISA Test is an enzyme-linked immunosorbent assay (ELISA) test designed for the qualitative detection of IgM antibodies to HAV in human serum.

ASSAY PRINCIPLE

The wells of HAV IgM ELISA test are coated with HAV recombinant antigen. Patient serum and Horse Radish Peroxidase conjugated with anti-human IgM recombinant antigens are added and incubated in the wells at 37°C. The HAV IgM antibodies, if present, bind to the solid phase and HRP conjugate simultaneously. All unbound antibodies and unbound HRP conjugate is washed off. Upon addition TMB substrate, the bound enzyme generates color. The intensity of the color is directly proportional to the concentration of anti HAV IgM antibodies in the samples.

SPECIMEN COLLECTION & PREPARATION

Collect blood by venipuncture. Allow to clot and separate the serum by centrifugation. If samples cannot be assayed immediately, they must be stored at 2-8°C or frozen.

REAGENTS

Materials provided with the kit

1. Eight x 12 well strips coated with HAV recombinant antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Negative Control
3. Positive Control
4. HRP conjugate (6 ml)
5. Substrate (TMB 11 ml)
6. Stopping solution (11 ml)
7. Wash buffer 30 x (25 ml)

Materials required but not provided

Microtiter plate reader capable of measuring optical density (OD) at 450 nm either with or without a reference filter of 620-690 nm. Micropipettes capable of delivering 5-200 µl, pipette tip and deionized or distilled water.

REAGENT PREPARATION

1. Bring all reagents to room temperature and gently mix well.
2. Dilute the wash buffer (30x) with deionized or distilled water. Mix well.

ASSAY PROCEDURE

1. Dispense 50µl of serum specimen, negative control, and positive control to the wells.
2. Cover the wells and incubate at 37°C for 45 minutes.
3. Wash the wells 5 x with diluted wash buffer.
4. Blot wells to dry.
5. Add 50 µl (1 drop) of HRP conjugate solution to each well and incubate at 37°C for 30 minutes.
6. Wash the wells by repeating step 3 and 4.
7. Add 100 µl (2 drop) of TMB substrate solution to each well and incubate for 10 minutes at 37°C. (Note: Do not mix TMB with other solutions for use. Use TMB solution only for this step.)
8. Add 50 µl (1 drop) of stop solution to each well. Gently shake wells.
9. Set the microplate reader wavelength at 450 nm. Measure the OD of each well. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

RESULTS

The presence or absence of HAV IgM is determined by comparing the absorbance of the specimens with Cutoff Value of the test. The Cutoff Value for HAV IgM ELISA is calculated as 0.1 + the mean Absorbance of the negative control (if the mean value of negative is <0.050, Cutoff Value = 0.100 + 0.05= 0.150)

INTERPRETATION

	Specimen OD ratio
Negative	< Cutoff Value
Positive	≥ Cutoff Value

The negative result indicates that there is no detectable anti HAV IgM antibodies in the specimen while positive result reveals that the patient might currently have been infected by HAV.

QUALITY CONTROL

The mean OD value of the positive controls deducts mean OD value of the negative control should be greater than 0.300. If not, the test should be considered invalid and should be repeated.

EXPECTED RANGES OF VALUES

Assay reproducibility was determined by assaying 3 positive specimens in replicates of 10 on 2 consecutive runs using the same production lot. The coefficient of variation (%CV) of Intra-assay and Inter-assay were calculated.

Table shows reproducibility of assay results:

Sample	Number of Tests	Intra Assay Precision %CV	Inter-Assay Precision %CV
1	10	7.3	6.5
2	10	6.9	8.2
3	10	7.4	7.7

PERFORMANCE CHARACTERISTICS

The sensitivity and specificity were evaluated at two external clinical sites. A total of 248 patient sera with acute HAV symptoms were run. Eighty-two were tested positive and 166 negative. The results agreed with final clinical find-out. Both sensitivity and specificity were 100%.
No cross reactivity was observed with specimen from patients infected with HIV, HBV, HCV, and HTLV. Additional studies of potentially interfering diseases (rheumatoid arthritis, auto-immune or viral diseases: 64 samples tested) have shown no cross reactions.

STORAGE CONDITIONS

Store the kit at 2-8°C. Keep the microwell strips sealed with desiccants in the aluminum bag. All kit components are stable until the expiration date printed on the label if the recommended storage conditions are strictly followed.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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