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Chlamydia Trachomatis IgM ELISA Kit

(Catalog # E4713-100; 96 assays; Storage at 4ºC)

I. Introduction:

Chlamydia Trachomatis is one of the most common human pathogens. Of the 15 recognized serotypes, 4 (A, B, Ba, and C) have been shown to cause hyperendemic blinding trachoma, a disease which afflicts hundreds of millions of people in developing countries. Three serotypes (L-1, L-2, and L-3) are the causes of lymphogranuloma venereum (LGV), a sexually transmitted systemic disease. BioVision's Chlamydia Trachomatis ELISA Kit can detect LGV type 2 broadly reacting antigen of Chlamydia Trachomatis. It will detect Chlamydia Trachomatis, Chlamydia Psittaci and Chlamydia Pneumoniae (TWAR) antibodies. Purified Chlamydia Trachomatis antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the Chlamydia Trachomatis IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic Substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

II. Applications:

- This ELISA kit is used for the determination of specific IgM antibody to Chlamydia in human serum samples

III. Sample Type:

- Serum

IV. Kit Contents:

Components	E4713-100	Part Number
Microwell strips	12X 8 strips	E4713-100-1
Absorbent Solution	22 ml	E4713-100-2
Calibrator	150 µl	E4713-100-3
Negative Control	150 µl	E4713-100-4
Positive Control	150 µl	E4713-100-5
Wash Buffer (20X)	50 ml	E4713-100-6
Enzyme Conjugate	12 ml	E4713-100-7
TMB Chromogenic Substrate	12 ml	E4713-100-8
Stop Solution	12 ml	E4713-100-9

V. User Supplied Reagents and Equipment:

- Distilled or deionized water
- Microplate reader capable of measuring absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:

The entire kit may be stored at 4°C
Wash buffer: Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 20X wash buffer to a final volume of 1 liter.
Bring all sampless and kit reagents to room temperature (20-25 °C) and gently mix.

VII. Assay Protocol:

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the samples, negative control, positive control and calibrator to 200 µl of absorbent solution. Mix well.
3. Add 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the blank, add 100 µl adsorbent solution in well. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Add 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Add 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop solution to stop reaction. Note: Make sure there are no air bubbles in each well before reading.
9. Read O.D. at 450 nm with a microwell reader.

VIII. Calculation:

To obtain Cut off OD value: Multiply the OD of Calibrator by Factor (f) printed on label of Calibrator. Calculate the IgM Index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

For example:
If Factor (f) value on label = 0.4
This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.

Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition IgM Index = 1)
Patient sample O.D. = 0.580
IgM Index = 0.580 / 0.44 = 1.32 (Positive result)
Patient sample O.D. = 0.320
IgM Index = 0.320 / 0.44 = 0.73 (Negative result)

Quality Control:
The test run may be considered valid provided the following criteria are met:
1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The IgM Index for Negative and Positive Control should be in the range stated on the labels.

Interpretation:
Negative: IgM Index of 0.90 or less are seronegative for IgM antibody.
Equivocal: IgM Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: IgM Index of 1.00 or greater.

Limitation of the procedure:
1. A single serum sample cannot be used to determine recent infection.
2. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgM antibody and render an IgM
3. Index result negative.
4. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Performance Characteristics:
Precision:
The precision of the assay was evaluated by testing three different sera eight replicates on 3 days. The intra-assay and inter-assay C.V. are summarized below:

N=8	Negative	Low positive	Positive
Intra-assay	12.5%	10.2%	9.5%
Inter-assay	15.4%	12.5%	10.6%

Cross-reactivity:
A study was performed to determine the cross-reactivity of the test to the following antibodie:
1. IgM of EBV, Mumps, Measle, and VZV.
2. IgM of Rubella, Toxo, CMV, HSV 1, and HSV 2.

All positive samples tested give negative results.

Related Products:
Chlamydia Trachomatis IgG ELISA Kit (E4712)
Chlamydia Trachomatis IgA ELISA Kit (E4711)

FOR RESEARCH USE ONLY! Not to be used on humans.