Version 04-05/24

User's Manual

Echovirus IgM ELISA Kit

REF **DEIA-NS2401-11**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

ELISA classic Echovirus IgM are quantitative and qualitative tests for detection of human antibodies in serum or plasma against Echovirus. For sale in the U.S. for Research Use Only. Not for use in diagnostic procedures.

General Description

Echoviruses (31 serotypes) and Coxsackieviruses A (23 serotypes) and B (6 serotypes) belong to the family of Picornaviridae, genus Enterovirus. Furthermore, this genus includes three poliovirus serotypes and Enterovirus types 68-71. Typical for this virus group are the same physical, chemical and biological properties and a similar epidemiologic pattern.

Enteroviruses are ubiquitous pathogens that are mainly transmitted by faecal-oral route as well as by aerosol (droplet infection). Human to human transmission occurs via dirt or smear infection. The portal of entry of enteroviruses is the respiratory tract (especially the oropharynx) and the intestine, where they infect and multiply in the oropharyngeal and intestinal epithelium. From there, the virus can be carried by the bloodstream to the cells of the reticulo-endothelial system and certain target tissues such as myocardium, meninges and skin.

While in tropical regions there is no apparent seasonal occurrence of Enterovirus infections, in temperate countries infections occur most frequently in summer and subside in autumn.

Echovirus infections can be confirmed by virus isolation and identification, serologically, or by PCR. ELISA techniques have been introduced in recent years for serodiagnostics. Due to the large number of Echovirus serotypes, assays showing a broad Echovirus reactivity are useful. Broad reactivity is achieved by using heat denatured virions as antigens.

The CD ELISA classic Echovirus, highly purified, heat-treated Echoviruse types 6 and 9 are bound to the solid phase.

Principles of Testing

Microtiter wells are coated with antigens. This constitutes the solid phase. Sample is added to the wells and any antibodies specific for the antigen present will bind to the solid phase. After removal of unbound material, anti-human IgM conjugated to an enzyme (alkaline phosphatase) is allowed to react with the immune complex. After removal of excess conjugate by washing, an appropriate substrate (paranitrophenylphosphate) is added, with which the conjugated enzyme reacts producing a colored derivative of the substrate. The color intensity is proportional to the level of specific antibody bound and can be quantified photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with 8 antigen coated single wells (altogether 96), 1 frame, the coating material is inactivated. 12

2. Standard serum (ready-to-use), Human serum in phosphate buffer with protein; negative for anti-HIV-Ab, anti-HBs-Ag (Hepatitis B-Virus-surface antigen) and anti-HCV-Ab; preservative: < 0.1% sodium azide, colouring: Amaranth O. 2 × 2 ml

3. Negative control serum (ready-to-use), Human serum in phosphate buffer with protein; negative for anti-HIV-Ab, anti-HBs-Ag (Hepatitis B-Virus-surface antigen) and anti-HCV-Ab; preservative: < 0.1% sodium azide, colouring: Lissamine green V. 2 ml

4. Anti-human-IgM-conjugate (ready-to-use), Anti-human-IgM from goat (polyclonal), conjugated to alkaline phosphatase, stabilized with protein stabilization solution, preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane. 13 ml

5. Washing solution concentrate (sufficient for 1000ml), Sodium chloride solution with Tween 20, 30 mM Tris, preservative: < 0.1% sodium azide. 33.3 ml

6. Dilution buffer, Phosphate buffer with protein and Tween 20; preservative: < 0.1% sodium azide, 0.01 g/l Bromphenol blue sodium salt. 2 × 50 ml

7. Stopping solution, 1.2 N sodium hydroxide, 15 ml

8. Substrate (ready-to-use), Para-nitrophenylphosphate, solvent free buffer, preservative: < 0.1 % sodium azide, (Substrate in unopened bottle may have a slightly yellow coloring. This does not reduce the quality of the product!). 13 ml

Materials Required But Not Supplied

1. common laboratory equipment
2. for the IgM-ELISA
3. photometer for microtiter plates with filter, wave length 405 nm, recommended reference wave length 620 nm - 690 nm (e.g. 650 nm)
4. incubator 37°C
5. moist chamber
6. distilled water

Storage

Reagent	Storage	Stability
microtiter strips (antigen)	after opening at 2-8°C in closed aluminum bag with desiccant. Strips which are not used must be stored in the press-seal bag of aluminum compound foil under dry and airtight conditions!	4 weeks
control sera / standard sera	after opening at 2-8°C	until expiry date; 24 months from date of production
conjugate	ready-to-use solution, at 2-8°C, Avoid contamination (sterile tips!)	until expiry date 28 months from date of

		production
washing solution	concentrate after opening at 2-8°C; working dilution at 2-8°C; working dilution at room temperature. Bottles used for the working dilution should be cleaned regularly, discard cloudy solutions.	until expiry date; 2 weeks; 1 week
dilution buffer	after opening at 2-8°C, Discard cloudy solutions!; unopened	24 months; until expiry date; 36 months from date of production
substrate	ready-to-use solution at 2-8°C, protected from light! Avoid contamination (sterile tips!) Discard when solution turns yellow (extinction against distilled water > 0.25).	until expiry date 24 months from date of production
stopping solution	after opening at room temperature	until expiry date

Specimen Collection And Preparation

1. Sample preparation and storage

Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found. Obviously contaminated samples (serum or plasma) should not be tested due to the risk of wrong results.

Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples.

Samples must not be thermally inactivated.

2. Sample preparation

Rheumatoid factors are autoantibodies mainly of the IgM-class, which preferably bind to IgG-immune-complexes. The presence of non-specific IgM-antibodies (rheumatoid factors) can lead to false-positive results in the IgM-assay. Furthermore, the possibility exists, that weak-binding pathogen-specific IgM-antibodies are displaced by stronger-binding IgG antibodies.In this case, IgM-detection can lead to false-negative results. Therefore it is necessary to pretreat samples with rheumatoid factor-absorbens prior to IgM detection.

Before running the test, rheumatoid factor-absorbent (V_1) must be diluted 1+4 in dilution buffer (V_2).

$V_1 + V_2 = 1 + 4$	add	200 µl	Rf-absorbent
	each to 800 µl		dilution buffer

Samples (V_4) must be diluted in this Rf-dilution buffer (V_3)

$V_4 + V_3 = 1+100$	add	10 µl	sample
	each to	1000 µl	Rf-dilution buffer

3. Sample storage

The stoppered samples can be stored in a refrigerator up to 7 days at 2-8°C. Extended storage is possible at ≤ -20°C.

Avoid repeated freezing and thawing of samples.

Diluted samples can be stored at 2-8°C for one week.

Reagent Preparation

1. Microtest strips
Microtest strips in frame are packed with desiccant in an aluminum bag. Take unrequired cavities out of the frame and put them back into the press-seal bag. Close press-seal bag carefully to ensure airtight conditions.

2. Control sera / standard sera
Control and standard sera are ready-to-use and must not be diluted any further. They can be used directly for the test run.
For each test run and for each test system - independent of the number of microtest strips to be used - control and standard sera must be included. The cut-off-control should be set up in duplicate. With the quantitative tests the standard serum should also be set up in duplicate. Do not treat control sera with Rf-absorbent.
3. Anti-human-IgM-AP-conjugate (ready-to-use)
Please do not mix up conjugates from different kits. They are optimized for each lot. Avoid contamination of ready-to-use conjugates (please pour sufficient for test into a secondary container to avoid repeatedly pipetting from the original bottle).
4. Washing solution
Dilute washing buffer concentrate (V_1) 1:30 with distilled water to a final volume of V_2.
Example:

buffer concentrate (V_1)	final volume (V_2)
33.3 ml	1000 ml
1 ml	30 ml

5. Dilution buffer for samples (ready-to-use)
6. Substrate (ready-to-use)
To avoid contamination use gloves. For pipetting substrate solution use sterile tips only!
7. Stopping solution (ready-to-use)

Assay Procedure

1. Place the required number of cavities in the frame and prepare a protocol sheet.
2. Add each 100 µl of diluted sample or ready-to-use controls into the appropriate wells of microtest strips. Spare one well for substrate blank, e.g.:

well A1: substrate blank

well B1: negative control

well C1: standard serum

well D1: standard serum

well E1: sample 1....

3. Sample incubation for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber
4. After incubation wash all wells with washing solution (by automated washer or manually):

   aspirate or shake out the incubation solution

   fill each well with 300 µl washing solution

   aspirate or shake out the washing buffer

   repeat the washing procedure 3 times (altogether 4 times!)

   dry by tapping the microtest plate on a paper towel
5. Addition of conjugate: Add 100 µl of IgM-conjugate (ready-to-use) to the appropriate well (except substrate blank)
6. Conjugate incubation for 30 minutes (+/- 1 min)* at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above)
8. Addition of substrate: Add 100 µl substrate solution (ready-to-use) to each well (including well for substrate blank!)
9. Substrate incubation for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.
10. Stopping of the reaction: Add 100 µl stopping solution to each well, shake microtest plate gently to mix.
11. Read optical density: Read OD within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

***Please note, that under special working-conditions internal-laboratory adaptations of the incubation times could be necessary.**

Calculation

1. Single-point quantification with the 4PL method

Optimized assignment of extinction signals to quantitative values is guaranteed by using non-linear functions, which adjust a sigmoide curve without any further transformation to OD-values.

Determination of antibody concentrations with the ELISA is carried out by the logistic-log-model (4 PL; 4 parameter) which is ideal for exact curve-fitting. It is based on the formula:

$$OD = A + \frac{D - A}{1 + e^{B(C - \ln conc.)}}$$

The parameters A, B, C, and D are representative for the exact shape of the curve:

1. lower asymptote → parameter A

2. slope of the curve → parameter B
3. turning point → parameter C
4. upper asymptote → parameter D

For each lot the standard curve is evaluated by CD in several repeated test runs under optimal conditions. Time consuming and cost intensive construction of the standard curve by the user is not necessary.

For evaluation of antibody concentrations a lot specific standard curve as well as a lot specific evaluation table is included with each test kit. Appropriate evaluation software is available on request.

To compensate for normal test variations and also for test run control a standard serum is used in each individual test run. For this control serum a "reference value" with a validity range is determined by the quality control of the producer. Within this range a correct quantification of antibody concentration is ensured. Since the standard serum is not necessarily a positive control, the value of the standard serum may be borderline or negative in some ELISA tests.

2. Criteria of validity

a. the substrate blank must be OD < 0,25

b. the negative control must be negative

c. quantitative ELISA: the mean OD-value of the standard serum must be within the lot specific validity range given on the quality control certificate of the kit (after subtraction of the substrate blank!)

d. qualitative ELISA: the OD-value of the positive control must be within the validity range, which is given in the lot specific quality control certificate of the kit (after subtraction of the substrate blank!)

e. the variation of OD-values may not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

3. Calculation quantitative

a. Non-automated evaluation

For the test evaluation a standard curve and an evaluation table are included in the test kit so that the obtained OD-values may be assigned to the corresponding antibody activity.

The reference value and the validity range of the standard serum is given on the evaluation table (quality control certificate).

The blank (A1) must be subtracted from all OD-values prior to the evaluation.

Method 1: Qualitative Evaluation

To fix the cut-off ranges please multiply the mean value of the measured standard-OD with the numerical data of the certificate of quality control (see special case formulas), e.g.:

OD = 0.502 x MW(STD) with upper cut-off

OD = 0.352 x MW(STD) with lower cut-off

If the measured mean absorbance value of the standard serum is 0.64, the range of the cutoff is in between 0.225-0.321.

Method 2: Continuous determination of antibody activities using the standard curve.

So called interassay variations (day to day deviations and laboratory to laboratory deviations) are

compensated by multiplication of the current measured value obtained with a sample with the correction factor F. This factor is calculated as follows:

$$F = \frac{\text{OD-reference value (of standard serum)}}{\text{OD-current value (of standard serums)}}$$

The procedure is necessary to adjust the current level of the test of the user with the lotspecific standard curve.

First, daily deviations have to be corrected by calculating a factor (correction factor F):

1. The mean of the two OD-values of the standard serum has to be calculated and checked that it is within the given validity range.
2. Calculation of the factor "F": the given reference value is divided by the mean of the extinction of the standard serum:

   F = reference value extinction standard serum / mean value extinction standard serum.
3. All measured values of samples are multiplied by "F".
4. Antibody activities in IU/ml or U/ml can be determined from the standard curve with the corrected values.

Precautions

1. Evidence of deterioration

Only use ELISA reagents for test procedure, since all reagents are matched. In particular standard and control sera are defined exclusively for the test kit to be used. Do not use them in other lots.

There are three different conjugate concentrations for each immunoglobulin class: LOW, MEDIUM, HIGH

The classification is written on each label as follows:

e.g. IgG + lowly concentrated IgG conjugate

IgG ++ medium concentrated IgG conjugate

IgG +++ highly concentrated IgG conjugate

In rare cases the use of special conjugate is necessary to guarantee consistent quality for our products. Special conjugates are produced in a separate lot and do not wear the "+" sign. Therefore, special conjugates are not exchangeable with other conjugates.

Please pay close attention to notifications on labels!

Unopened, all components of the ELISA kits may be used up to the dates given on the labels, if stored at +2°C to +8°C. Complete stability and storage data are described under "Storage".

Each reagent has been calibrated and optimized for the test. Dilution or alteration of these reagents may result in a loss of sensitivity.

Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed to avoid evaporation and contamination with microorganisms since incorrect test results could occur due to interference from proteolytic enzymes.

To open the press-seal bag please cut off the top of the marked side, only. Do not use the strips if the aluminum bag is damaged or if the press-seal bag with remaining strips and desiccant was not properly

closed.

Bring all reagents to room temperature before testing.

Use aseptic techniques for removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results ensure not to contact or sprinkle the top-walls of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.

Reproducibility is dependent on thorough mixing of the reagents. Shake the flasks containing control sera before use and also all samples after dilution (e.g. by using a monomixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures.

Significant time differences between pipetting the first and last well of the microtiter plate when filling samples/control sera, conjugate or substrate may result in different "pre incubation" times, which may influence the precision and reproducibility of the results.

Optimum results can only be achieved if ELISA instructions are followed strictly.

The test is not valid, if the lot-specific validation criteria on the quality control certifcate are not fulfilled.

Inadequate washing will affect the test results:

The washing procedure should be carried out carefully. If the washing procedure is carried out automatically follow the instruction manual of the respective washer. Flat bottom wells are used for ELISA. All wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer by tapping the inverted microtest plate on a paper towel. Avoid foam! Do not scratch coated wells during washing and aspiration. If using an automated washer, ensure it is operating correctly.

2. Statements of warning

The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

All kit reagents and human specimens should be handled carefully, using established good laboratory practice.

a. This kit contains human blood components. Although all control- and cut-off-sera have been tested and found negative for HBs-Ag-, HCV- and HIV-antibodies, they should be considered potentially infectious.

b. Do not pipette by mouth.

c. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

d. Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.

e. Samples and other potentially infectious material should be decontaminated after the test run.

f. Reagents should be stored safely and be unaccessible to unauthorized access e.g. children.

g. Stopping solution: corrosive (C); causes acid burn (R34) use safety glasses, gloves and laboratory coat while handling!