Version 15-12/24

User's Manual

Respiratory Syncytial Virus IgM ELISA Kit

REF **DEIA373M**

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

The Respiratory Syncytial Virus (RSV) IgM tests are immunoassays for detection of human antibodies in serum or plasma against Respiratory Syncytial Virus. For sale in the U.S. for Research Use Only. Not for use in diagnostic procedures.

General Description

Respiratory Syncytial Virus (RSV) causes upper and lower respiratory tract infections predominantly in infants and children. Such infections are responsible for bronchitis as well as pneumonia and may be life threatening. In addition, an RSV infection may often result in middle ear inflammation. In older children and adults, an infection is usually associated with mild symptoms although the elderly may develop more sever symptoms.

RSV is a pleomorphic RNA virus with a diameter of around 150-300nm. Originally isolated from chimpanzees, the firus acquired its name from the ability to form syncytia when cell cultures are infected with the virus.

Infections are transmitted from host to host through contact with mucous membranes of the eyes, mouth, or nose. Epidemiological studies have shown that all children by the age of 2 years have already experienced an RSV infection. Immunity developed as a result of infection is poor due to the virus spreading by syncytia formation. A protective immunity is primarily due to antibody responses to the viral surface glycoproteins G and F, whereby the F protein stimulates a humoral as well as a cellular immune response.

Principles of Testing

THE ELISA (Enzyme Linked Immnosorbent Assay) is an immunoassay that is particularly suited to the detection of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies are present in a sample, they bind to the fixed antigen. A secondary antibody that has been conjugated with the enzyme alkaline phosphatase detects and binds to the antigen-antibody complex. The colorless substrate p-nitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of antibody in the sample and is measured photometrically.

Reagents And Materials Provided

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

2.Cut-off serum (ready-to-use) C/O, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: <0.1% sodium azide; coloring: Chinaldin yellow. 2×2ml

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

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

5. Anti-human-IgM conjugate (ready-to-use) APC, Anti-human-IgM polyclonal antibody, conjugated to alkaline phosphatase, stabilized with protein stabilization solution; preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane. 13 ml

6. Washing solution concentrate (sufficient for 1000ml) WASH, Sodium chloride solution with Tween 20 and 30mM Tris-HCl, pH 7.4; preservative: < 0.1% sodium azide. 33.3 ml

7. Dilution buffer (ready-to-use) DILB, Protein-containing phosphate buffer with Tween 20; preservative: < 0.1% sodium azide; coloring: 0.01g/l Bromphenol blue. 2×50 ml

8. Stopping solution (ready-to-use) STOP, <0.1N sodium hydroxide, 40mM EDTA. 15ml

9. Substrate (ready-to-use) pNPP, Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide. 13 ml

Materials Required But Not Supplied

1. Common laboratory equipment
2. For the IgM detection: SERION Rf-Absorbent (Order no. Z200/20ml)
3. Photometer for microtiter plates with filter, wave length 405 nm, recommended reference wave length 620 nm - 690 nm (e.g. 650 nm)
4. Microtiter plate washer
5. Incubator 37°C
6. Moist chamber
7. Distilled water

Storage

Microtiter strips (coated with antigen): Unopened, See expiry date. After opening at 2-8°C in closed aluminum-bag with desiccant, Minimum shelf-life: four weeks.

Specimen Collection And Preparation

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

1. Interference by rheumatoid factors

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 falsepositive results in the IgM assay. Furthermore, the possibility exists that weak-binding pathogenspecific

IgM antibodies are displaced by stronger-binding IgG antibodies leading to a false-negative IgM result. Therefore it is necessary to pretreat samples with rheumatoid factor-absorbent prior to IgM detection (Rf-

Absorbent). Rf-absorption is performed by incubation of the sample in Rf-dilution buffer for 15 minutes at room temperature or overnight at 4°C.

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

$V_1 + V_2 = V_3$ (1:4)	add	200ul	Rf-absorbent
	each to	800ul	dilution buffer

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

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

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

2. Sample Storage

Samples should not be stored for more than 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

Bring all reagents to room temperature before using.

1. Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do no use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

2. Control Sera / Standard Sera (ready-to-use)

Control and standard sera are ready-to-use and must not be diluted any further. For each test run - independent of the number of microtest strips to be used - control and standard sera must be included. Standard and cut-off sera should be set up in duplicate. Do not treat control sera with Rf absorbent.

3. Anti-human-IgM AP-Conjugate (ready-to-use)

The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label.

4. Washing Solution (Concentrate)

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)

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product!

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.:

Qualitative ELISA
Substrate blank
Negative control
Cut-off serum
Cut-off serum
Positive control
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 300µl 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 100µl of the ready-to-use IgM conjugate to the appropriate wells (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 100µl of ready-to-use substrate solution 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 100µl stopping solution to each well, shake microtest plate gently to mix.
11. Read optical density: Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wave length between 620nm and 690nm (e.g. 650nm).

For the periodic verification of the test method, in order to fulfill the requirements of laboratory internal quality management systems, we recommend using controls to determine precision and accuracy of test runs. The use of controls is described in specific instruction manuals.

Calculation

For the evaluation of test runs a lot-specific quality control certificate with declarations concerning cut-off

serum and positive control is included in every SERION ELISA classic.

Before evaluation the blank value (blank) has to be subtracted from each sample value. For determination of the cut-off range in OD the mean of the readings for the cut-off serum has to be calculated. The cut-off range in OD corresponds to the mean value of the cut-off serum +/- 10%.

OD sample	more than	10% over	OD cut-off	positive
OD sample	+/-	10% of	OD cut-off	borderline
OD sample	more than	10% under	OC cut-off	negative

Interpretation Of Results

Criteria of Validity

The substrate blank must be <0.25 OD.

The negative control must be negative.

By use of qualitative tests the OD value of the positive control and the mean OD value of the cut-off serum must be within the validity ranges which are given on the lotspecific quality control certificate of the kit (after subtraction of the substrate blank!)

The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

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

Precautions

Assay Notes:

Optimum results can only be achieved if the instructions are strictly followed. Only use ELISA reagents when using ELISA immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of ELISA immunoassays are defined exclusively for the test kit to be used and must not be used in other lots. Washing solution, substrate, and stop solution can be used for all ELISA immunoassays irrespective of the lot and the test.

Each ELISA test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results. The dilution buffers can be used irrespective of the lots.

There are three different conjugate concentrations for each immunoglobulin class (IgG) indicated on the label as + (low), ++ (medium), and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other ELISA immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the tubes containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

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 dispensing samples and control sera, conjugate, or substrate can result in different pre-incubation times which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.

Safty Notes:

The ELISA is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and samples should be handled carefully using established good laboratory practice.

This kit contains human blood components. Although all control and cut-off sera have been tested and found negative for anti-HIV Ab, HBs-Ag (Hepatitis B Virus surface-Antigen) and anti-HCV-Ab, they should be considered potentially infectious.

Do not pipette by mouth.

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

Wear disposable glove, laboratory coat and safety glasses while handling kit reagents or samples.

Wash hands thoroughly afterwards.

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

Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.