



**Mumps IgM capture EIA**  
An Enzyme Immunoassay for the detection of mumps virus specific IgM  
in human serum / plasma samples

**Cat. No: MuVM004**

For *in-vitro* diagnostic use.

**IVD**



104A High Street, Brentford, Middlesex, TW8 8AT, U.K.

Phone: 1-44-020 8230 8777

Fax: 1-44-020 8230 8778

Email: [customerservice@microimmune.co.uk](mailto:customerservice@microimmune.co.uk)

[orders@microimmune.co.uk](mailto:orders@microimmune.co.uk)

DISTRIBUTED IN THE U.S.A. FOR MICROIMMUNE LIMITED BY:

**BluePoint Bioscience**

10075 Tyler place #9

Ijamsville, MD U.S.A. 21754

Tel: 00 1 240-246-4912

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## INTENDED USE AND APPLICATION

Enzyme Immunoassay (EIA) for the detection of human IgM antibodies to mumps virus in serum / plasma specimens. This product is for *in-vitro* diagnostic use by trained laboratory personnel.

## SUMMARY AND EXPLANATION

Mumps is an acute contagious viral disease caused by mumps virus, a member of the genus rubulavirus in the family Paramyxoviridae. The disease is usually mild with asymptomatic infections occurring in 15-20% of infected individuals. Mumps usually presents as parotitis about 16 to 18 days after infection. Symptoms include fever, headache, malaise, myalgia and anorexia. Orchitis affects up to 38% of post-pubertal men with mumps. Aseptic meningitis occurs in approximately 10% of patients and mumps meningoencephalitis is seen in a small number (0.25%) of mumps cases (1).

Mumps is transmitted through saliva by direct contact or through droplet spread. Transmission can occur one or two days before the onset of parotitis and up to three days after parotitis has subsided.

Epidemiological surveys have indicated that the incidence of mumps disease is dramatically reduced in countries where vaccination coverage, practised as either single mumps vaccine, a combination measles and mumps vaccine (MM) or the measles, mumps and rubella vaccine (MMR), is high (2).

Antibody responses after mumps infection or vaccination are predominantly to the nucleoprotein (NP) and the haemagglutinin-neuraminidase (HN) of mumps virus. Detection of IgM antibodies to NP has been shown to be useful in diagnosing recent mumps infections (3-4).

## TEST PRINCIPLE

In the Microimmune Mumps IgM capture EIA diluted serum / plasma is added to anti-human IgM coated microtitre wells. IgM in the specimen binds to the wells and after washing, recombinant mumps nucleoprotein (rMuNP) antigen is added. Mumps specific IgM in the sample, if present in the sample, binds the rMuNP. After washing the wells, a monoclonal antibody to the rMuNP conjugated to horseradish peroxidase is added. After washing, tetramethylbenzidine (TMB) substrate is added to reveal the presence of specific IgM. The presence of mumps specific IgM results in a colour change in the TMB from colourless to blue and then yellow on terminating the enzymatic reaction. The colour change and intensity are monitored using a spectrophotometric plate reader set at 450nm with a correction filter between 620 and 650nm. The presence of mumps specific IgM is indicated by optical density values above the cut-off.

## MATERIALS PROVIDED

Each kit contains sufficient materials for 96 tests.

1. ANTI-HUMAN IgM PLATE PN 2103: 8x 12 microwell strips coated with anti-human IgM antibody in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edges and separating the re-sealable joint. Return unused strips to the pouch with desiccant and store at 2-8°C. Strips must be used within 3 months of initial opening.
2. SERUM DILUENT (100 mL) **PN 2040**: One bottle containing phosphate buffered saline, protein stabilizer, detergent and red dye.
3. WASH BUFFER, 10x (100 mL) **PN 2024**: One bottle containing 10x phosphate buffered saline, detergent and preservative. Dilute 1 in 10 with good quality deionized or distilled water.
4. POSITIVE CONTROL (0.6mL Mumps IgM EIA only) **PN 2141**: One vial containing pre-diluted serum positive for mumps IgM antibody in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to use. DO NOT DILUTE.**
5. NEGATIVE CONTROL (1.5 mL Mumps IgM EIA only) **PN 2142**: One vial containing pre-diluted serum negative for mumps IgM antibody in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to use. DO NOT DILUTE.**
6. 100 x rMuNP Antigen (0.10 mL) **PN 2136**: One vial containing 100 x concentrated recombinant mumps nucleoprotein antigen in phosphate buffered saline containing protein stabilizers, detergent and antimicrobial agent. **DILUTE IN ANTIGEN DILUENT BEFORE USE.**
7. CONJUGATE (10 mL, Mumps IgM EIA only) **PN 2139**: One vial containing anti-mumps NP IgG antibody conjugated to horseradish peroxidase (HRP) in a buffered solution containing protein stabilizers, detergent, antimicrobial agent and blue dye. **Ready to Use.**
8. TMB SUBSTRATE (10mL) **PN 2030a**: One vial containing 3,3',5,5' tetramethylbenzidine. **Ready to Use.**
9. STOP SOLUTION (10mL) **PN 2031a**: One vial containing 0.5M hydrochloric acid. **Ready to Use.**

10. ANTIGEN DILUENT (10mL, Mumps IgM EIA only) **PN 2137**: One Vial containing phosphate buffered solution with protein stabilizers, detergent, antimicrobial agent and yellow dye.

#### **MATERIALS REQUIRED BUT NOT PROVIDED**

- Good quality deionized or distilled water.
- Tubes suitable for diluting serum specimens and microtitre plate seals/ cover.
- Micropipettes and disposable tips capable of delivering 1000 µL, 100 µL, 10 µL and 5 µL volumes.
- Waste discard container with disinfectant.
- EIA plate reader capable of reading optical density at 450nm (and 620-650nm).
- Incubator, 37°C.

#### **REAGENT PREPARATION**

Dilute the 100x rMuNP Antigen ( PN 2136, Reagent 6) in Antigen Diluent (PN 2137, Reagent 10) before use. For example, add 10µL of the 100x rMuNP Antigen to 990µL of Antigen Diluent, **OR**, add the entire contents of the unused 100x rMuNP Antigen to the unused vial of Antigen Diluent and mix well. The diluted antigen should be orange in colour. The diluted antigen may be stored up to 7 days at 2-8°C.

Warm the WASH BUFFER 10x (Reagent 3) to re-dissolve any salts that may have formed on storage. Prepare working strength wash buffer by adding 1 part WASH BUFFER 10x to 9 parts distilled or deionized water. It is recommended that working strength buffer be prepared as required on the day of use. Remaining WASH BUFFER 10x should be stored at 2-8°C. Enough WASH BUFFER 10x has been provided to enable four washes of each well.

Bring all reagents to room temperature (18-25°C) prior to use.

#### **WARNINGS AND PRECAUTIONS**

- The POSITIVE CONTROL serum and NEGATIVE CONTROL serum are not reactive for antibodies to HIV 1 and 2, HCV or Hepatitis B surface antigen. The controls should be handled and disposed of as though potentially infectious.
- The TMB SUBSTRATE solution containing 3,3', 5,5'-tetramethylbenzidine has been reported to be non-carcinogenic. Contact with skin and mucous membranes should be avoided. Wear latex gloves when dispensing and using this reagent. If TMB SUBSTRATE comes into contact with skin and mucous membranes, rinse with copious amounts of water.
- The STOP SOLUTION contains hydrochloric acid (0.5M). Contact with skin and mucous membranes should be avoided. If the STOP SOLUTION comes into contact with these sites, rinse with copious amounts of water.
- Wear disposable gloves when handling clinical specimens and kit components. Treat all clinical specimens and controls and any materials coming into contact with them as potentially infectious.
- Dispose clinical material and potentially infected materials in accordance with local regulations.
- Do not mix components of one lot of kits with components from other lots.
- Avoid microbial contamination of reagents. Do not use reagents that show signs of contamination.
- Good laboratory procedure should be employed to avoid cross contamination of samples and reagents. Take out only the required volume of reagent from the original container (usually 0.9-1.0mL per strip) for dispensing into wells. Discard unused reagents - do not return to containers.

#### **SPECIMEN COLLECTION**

Handle all blood, serum and plasma as potentially infectious material.

Optimal performance is obtained with specimens taken between seven days and up to four weeks after onset of symptoms.

Serum and plasma (EDTA, citrated or heparinized) samples are suitable specimens for the test and should be obtained using standard procedure.

## STABILITY AND STORAGE

When stored at 2-8°C, the kit is stable up to the expiration date printed on the kit label.

## ENZYME IMMUNOASSAY PROCEDURE

1. Bring all reagents to room temperature (18-25°C) before use.
2. Dilute serum/plasma samples 1/201 in SERUM DILUENT (PN 2040, Reagent 2). Dispense 5 µL of specimen into a labelled tube and add 1mL of SERUM DILUENT.
3. Remove and assemble the required number of microwell strips from the ANTI-HUMAN IgM PLATE (PN 2103, Reagent 1) to perform the test. A minimum of 4 wells is needed for the controls which must be included in each test run. Return unused microwell strips and the desiccant to the foil pouch and reseal.
4. Pipette 100 µL/well of the POSITIVE CONTROL (PN 2141, Reagent 4) and NEGATIVE CONTROL (PN 2142, Reagent 5) controls to assigned wells. One well for the POSITIVE CONTROL and three wells for the NEGATIVE CONTROL.
5. Pipette 100 µL/well of the diluted serum specimens to assigned wells. Only test the number of samples, in a single test run, that can be dispensed within ten minutes. Alternatively use a low binding microtitre plate to pre-dispense samples and then transfer to a test plate using a multichannel pipette. Cover microtitre plate with lid or sealing tape and incubate at  $37 \pm 2^\circ\text{C}$  in a moist chamber for  $30 \pm 2$  minutes.
6. Wash wells four times with diluted wash buffer (see REAGENT PREPARATION). The wash cycle is carried out as follows: Aspirate the contents of the well and dispense 350 µL/well of diluted wash buffer, leave to soak for approximately 30 seconds and aspirate. Repeat the wash cycle three further times. Alternatively an automatic plate washer may be used. After washing, tap the wells dry on absorbent paper.
7. Pipette 100 µL/well of the diluted rMuNP antigen solution (orange in colour, see REAGENT PREPARATION) to the wells. This is best performed with a multichannel pipette. Cover plate and incubate at  $37 \pm 2^\circ\text{C}$  in a moist chamber for  $30 \pm 2$  minutes.
8. Wash the wells four times with wash buffer as in step 6.
9. Pipette 100 µL/well of the conjugate (PN 2139, Reagent 7, blue in colour) to the wells. This is best performed with a multichannel pipette. Cover plate and incubate at  $37 \pm 2^\circ\text{C}$  in a moist chamber for  $30 \pm 2$  minutes.
10. Wash wells four times with wash buffer as in step 6.
11. Pipette 100 µL/well of the TMB SUBSTRATE (PN 2030a, Reagent 8). This is best performed with a multichannel pipette. Incubate for  $10 \pm 1$  minutes at room temperature (18-25°C) protected from strong light.
12. Pipette 100 µL/well of the STOP SOLUTION (PN 2031a, Reagent 9). This is best performed with a multichannel pipette. The stop solution should be added using the same timing and sequence used to add the substrate solution.
13. Read optical density at 450nm (set the reference wavelength at 620, or between 615 and 650nm, if available on the plate reader) using an EIA plate reader within 10 minutes of adding the STOP SOLUTION.

## QUALITY CONTROL

The optical density  $OD_{450/620\text{nm}}$  of the POSITIVE CONTROL should be  $> 0.4$ . The  $OD_{450/620\text{nm}}$  value for each of the three Negative Control (NC) wells should be  $> 0.05$  and  $\leq 0.25$ .

## CALCULATIONS

Calculate the mean  $OD_{450/620\text{nm}}$  of the three Negative Control wells ( $\bar{x}_{\text{NC}}$ ). The  $OD_{450/620\text{nm}}$  values of the individual wells should not differ by more than 30% from the  $\bar{x}_{\text{NC}}$ . If one of the three  $OD_{450/620\text{nm}}$  values differs by more than 30%, it should be omitted and the mean value re-calculated.

## INTERPRETATION OF RESULTS

The following criteria are required for a specimen to be identified as mumps specific IgM Positive, Negative or Equivocal.

**Mumps specific IgM Positive:** OD<sub>450/620nm</sub> of specimen is  $\geq$  xNC x 3.5

**Mumps specific IgM Negative:** OD<sub>450/620nm</sub> of specimen is  $<$  xNC x 3.0

**Mumps specific IgM Equivocal:** OD<sub>450/620nm</sub> of specimen is  $\geq$  xNC x 3.0 and  $<$  xNC x 3.5

Interpretation of a mumps specific IgM positive result is that the patient is likely to have had recent exposure to mumps virus through vaccination (e.g. with MMR) or through contact with wildtype mumps virus. In areas of low mumps prevalence, an acute case of mumps should always be confirmed by other tests. For example, the use of Microimmune mumps IgG capture EIA to monitor seroconversion, PCR on clinical specimens such as oral fluid or throat swabs and virus isolation may aid diagnosis of acute mumps infection.

A negative mumps specific IgM result indicates that the patient is not acutely infected with mumps. However, IgM antibodies may not have developed in samples taken very soon after onset of symptoms.

A sample giving an equivocal result should be re-tested. If the equivocal status cannot be resolved on re-testing, follow up samples taken between 7 and 21 days after the initial sample should be tested in parallel with a further retest of the first sample. If an equivocal result is obtained on re-testing a follow up sample, it should be reported as mumps IgM negative.

## LIMITATIONS OF THE TEST

Microbiological contamination of the specimens may lead to erroneous results.

Some serum specimens with rheumatoid factor (RF) can give false positive results in the test. If RF is suspected, remove the RF using a commercially available RF absorbent and retest in the Microimmune Mumps IgM capture EIA.

The Microimmune Mumps IgM capture EIA detects antibodies specifically to mumps nucleoprotein antigen. Antibodies to other mumps virus proteins are not detected in this assay.

Patient's profile, epidemiological data and the test results should be considered in making a diagnosis.

## TEST PERFORMANCE

The performance of the Microimmune Mumps IgM capture EIA (MI) was evaluated on panels of sera received by a reference laboratory (Enteric, Respiratory and Neurological Virus Laboratory ERNVL, Health Protection Agency, Colindale, U.K.) for routine investigation and collected as part of sero-epidemiological studies. Serum samples received by the reference laboratory for mumps investigation had been tested by an IgM antibody capture radioimmunoassay (MACRIA) (5).

## EVALUATION OF MICROIMMUNE MUMPS IgM CAPTURE EIA ON SERUM SAMPLES

A total of one hundred and fifty sera were tested. These included 126 sera received in ERNVL for routine mumps investigation by MACRIA, 8 sera from the MMRV IgM Serum Standard Panel (consisting of two IgM positives for measles, mumps, rubella and varicella zoster; Quest Biomedical, U.K.), 8 parvovirus B19 IgM positive samples and 8 RF positive serum samples. The results are summarised below in table 1.

Table 1. Evaluation of mumps specific IgM by MACRIA and MI

MACRIA	Microimmune Mumps IgM Capture EIA			TOTAL
	POS	NEG	EQV	
POS	71	4**	0	75
NEG	3*	71		74
EQV			1*	1
TOTAL	74	75	1	150

\*RF positive samples. \*\* One of the 4 discordant samples was also RF positive.

There was good agreement between MACRIA and the MI with discordant results obtained for only seven of the 150 sera tested. Of these seven samples, three MACRIA negative, MI positive serum samples were RF positive and represent MI false positives. Of the four MACRIA positive, MI negative samples, one was an RF sample which was also positive in a parvovirus B19 IgM capture EIA. Two of these samples were from a 40 year old female with polyarthritis and a 37 year old male without clinical details, suggesting that these may be MACRIA false positives. The last discordant result was obtained on a sample from a 14-year-old male with clinical details given as "unilateral mass on the left side".

Sensitivity and specificity of MI test compared to MACRIA was 94.7% (95% CI 86.9% to 98.5%) and 95.9% (95% CI 88.6% to 99.2%) respectively.

## REFERENCES

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3. Gut JP, Spiess C, Schmitt S, Kirn A (1985) Rapid diagnosis of acute mumps infection by a direct immunoglobulin M capture enzyme immunoassay with labelled antigen. J. Clin. Microbiol. 21:346-352.
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## WARRANTY

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