Anti-Mumps Virus ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2630-9601 G	Mumps virus	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides semiquantitative or quantitative in vitro determination of human antibodies of the immunoglobulin class IgG against mumps virus in serum or plasma to support the diagnosis of mumps.

Application: The Anti-Mumps Virus ELISA (IgG) enables quantitative detection of IgG antibodies against mumps virus and is suitable for diagnosis of acute mumps virus infections. Alongside direct pathogen detection (PCR), a significant increase in the IgG titer in a follow-up sample can confirm an acute mumps virus infection, in particular in vaccinated persons, who often do not exhibit IgM antibodies. An assessment of the immune protection is not possible by means of serology, since IgG antibodies against mumps viruses do not correlate reliably with the immune protection or with the presence of neutralising antibodies.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with mumps antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

LOT Lot description	CE		Storage temperature
IVD In vitro diagnostic medical device			Unopened usable until

Modifications to the former version are marked in grey.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C and +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer.
For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working-strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working-strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.
Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C; protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

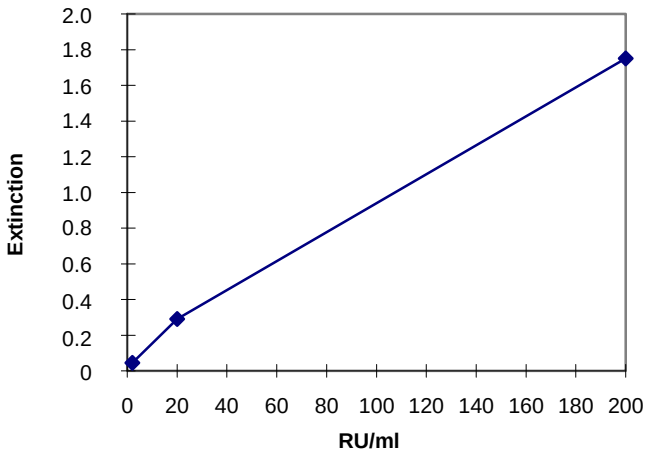
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken, is obtained by point-to-point plotting of the extinction readings measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the extinction of calibrator 1 (corresponding to 200 RU/ml), the result should be reported as “>200 RU/ml“. It is recommended that the sample be retested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by factor 4.
The upper limit of the normal range of non-infected persons (**cut-off**) recommended by EUROIMMUN is 20 relative units (RU)/ml. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations, the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant IgG titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against mumps virus, the calibration is performed in relative units (RU/ml).

For every group of tests performed, the extinction readings of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is provided by inactivated cell lysates of Vero cells infected with the "Enders" strain of mumps virus.

Linearity: The linearity of the Anti-Mumps Virus ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The Anti-Mumps Virus ELISA (IgG) is linear at least in the tested concentration range (4 RU/ml to 164 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Mumps Virus ELISA (IgG) is 0.3 RU/ml.

Cross reactivity: The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-Mumps Virus ELISA (IgG).

Antibodies against	n	Anti-Mumps Virus ELISA (IgG) positive
Adenovirus	9	0%
CMV	6	0%
EBV-CA	12	0%
Helicobacter pylori	8	0%
HSV-1	7	0%
Influenza A	12	0%
Influenza B	12	0%
Measles virus	12	0%
Mycoplasma pneumoniae	7	0%
Parainfluenza Pool	12	0%
Rubella	12	0%
RSV	11	0%
Toxoplasma gondii	4	0%
VZV	9	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	14	4.6
2	102	7.7
3	119	6.5

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	15	11.6
2	110	8.1
3	131	6.8

Sensitivity and specificity: 180 clinically pre-characterised patient samples (INSTAND, Labquality and NEQAS) were investigated with the EUROIMMUN Anti-Mumps Virus ELISA (IgG). The sensitivity amounted to 99.3%, with a specificity of 100%. Borderline results were not included in the calculation.

n = 180		INSTAND/Labquality/NEQAS		
		positive	borderline	negative
EUROIMMUN Anti-Mumps Virus ELISA (IgG)	positive	140	0	0
	borderline	3	1	0
	negative	1	0	35

Reference range: The levels of anti-mumps virus antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 77.2% of the blood donors were anti-mumps virus positive (IgG), wich reflects the known percentage of infections in adults.

Clinical significance

The mumps virus (MV) causes an acute, feverish general infection which occurs mainly in childhood and is very infectious. The infection is characterised by an inflammatory swelling of the parotid gland. Frequently involved are also pancreas, testes, ovaries and the central nervous system.

Postpubertal mumps in males not immunised as children is associated with a 40% incidence of orchitis. Clinicians should be aware that epididymo-orchitis may be secondary to mumps infection even with no history or clinical evidence of parotitis.

Mumps virus is a highly neurotropic virus with evidence of central nervous system invasion (CNS) in approximately half of all cases of infection. Hydrocephalus secondary to mumps is relatively rare. The mumps virus can be the aetiology of secondary autoimmune thrombocytopenic purpura. Additional complications of mumps are keratitis and/or iritis.

Antibodies against the mumps virus can be detected in the serum of nearly all patients after the illness has taken its course. Specific IgM and IgG antibodies to mumps virus are detected in sera of mumps patients by Enzyme Linked Immunosorbent Assay (ELISA) in agreement with the results obtained by Indirect Immunofluorescence Test (IFT). Mumps IgM antibodies can be demonstrated in all patients with serologically verified and clinically typical (parotitis, meningitis, or orchitis) mumps virus infection.

IgM antibodies develop soon after the onset of symptoms; most patients have IgM antibodies from the second day, and the highest titers are reached within the first week. The antibody response in mumps parotitis does not differ from that in mumps meningitis/encephalitis, while relatively higher antibody titers are found in patients with orchitis/epididymitis. Mumps IgM ELISA (also IgG) and IFT are more rapid and sensitive for the serological diagnosis of mumps infection than e.g. haemagglutination inhibition test (HI) and complement fixation (CF). Mumps virus infections often raise heterologous antibodies.

In mumps meninigitis/encephalitis, agent-specific antibodies of class IgG are produced in CSF. The intrathecal agent-specific antibody production is defined by the relative CSF/serum quotient CSQrel. (synonym: antibody specificity index). The quotient is calculated from the amount of agent-specific antibodies in total CSF IgG in proportion to the amount of agent-specific antibodies in total serum IgG.

Active immunisation of seronegative children is recommended in view of the complications which can develop. Neutralisation activity and persistence of antibodies are induced in response to vaccination of 12- to 24-month-old infants. Subsequently, antibody titers and neutralising activity can be determined using ELISA and IFT, with the highest prevalence at the earliest 120 days after vaccination. Test systems used both for monitoring the immunisation status and for serological diagnosis of the infection should be based on a combination of wild type and vaccination strain antigens. Life-long immunity is normally developed. However, antibody levels are 8 to 10 times lower in postvaccination sera than in convalescent sera.

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