Cytomegalovirus (CMV) IgG

NB-06-0438 (1x96 Tests)

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1. INTENDED USE

The NeoBiotech Cytomegalovirus IgG ELISA is intended for the qualitative determination of IgG class antibodies against CMV in human serum or plasma (citrate, heparin). This kit is intended for research use only. All information and results associated with this kit is to be used for research use only.

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgG-class antibodies against CMV is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with CMV antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured CMV-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of CMV specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

3. MATERIALS

3.1. Reagents supplied

▪ CMV Coated Wells (IgG): 12 breakapart 8-well snap-off strips coated with CMV antigen; in resealable aluminium foil.
▪ IgG Sample Diluent ***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
▪ Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
▪ Washing Solution (20x conc.)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.

▪CMV anti-IgG Conjugate**: 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG; coloured blue, ready to use; black cap.
▪ TMB Substrate Solution: 1 bottle containing 15 ml 3, 3', 5, 5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
▪ CMV IgG Positive Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
▪ CMV IgG Cut-off Control***: 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.
▪ CMV IgG Negative Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.

3.2. Materials supplied

▪ 1 Cover foil
▪ 1 Instruction for use (IFU)
▪ 1 Plate layout

3.3. Materials and Equipment needed

▪ ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
▪ Incubator 37°C
▪ Manual or automatic equipment for rinsing wells
▪ Pipettes to deliver volumes between 10 and 1000 µl
▪ Vortex tube mixer
▪ Deionised or (freshly) distilled water
▪ Disposable tubes
▪ Timer

4. STABILITY AND STORAGE

The reagents are stable up to the expiry date when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20…25°C) before starting the test run!

5.1. Coated Microplate

The breakapart snap-off strips are coated with CMV antigen. Store at 2...8°C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date.

5.2. Washing Solution (20xconc.)

Dilute Washing Buffer 1 + 19; e.g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25°C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

5.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8°C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away

6. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and and stored deep-frozen (-70 to -20°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.* Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

7.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any appreciable delays between the steps.
A clean, disposable tip should be used for dispensing each control and sample.
Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**

4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl CMV anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.** A blue color occurs due to the enzymatic reaction.
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
    Any blue colour developed during the incubation turns into yellow.
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

7.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.
If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable calculate the **mean absorbance values** of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1 : Absorbance value **< 0.100.**
- **Negative control** in B1: Absorbance value **< 0.200 and < cut-off**
- **Cut-off control** in C1 and D1: Absorbance value **0.150 – 1.30.**
- **Positive control** in E1: Absorbance value **> cut-off**.

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

8.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.44 + absorbance value Cut-off control 0.42 =0.86 / 2 = 0.43 Cut-off = 0.43

8.2.1. Results in NeoBiotech Units

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NeoBiotech-Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} =$ *37 NTU (NeoBiotech Units)*

Cut-off:	10	NTU
Grey zone:	9-11	NTU
Negative:	<9	NTU
Positive:	>11	NTU

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications. For further information about the specific performance, characteristics please contact NeoBiotech.

9.1. Precision

Interassay	**n**	**Mean (NTU)**	**Cv (%)**
#1	24	0,607	2,60
#2	24	1,359	10,63
#3	24	1,904	3,09
Intraassay	**n**	**Mean (OD)**	**Cv (%)**
#1	12	23,74	2,37
#2	12	32,82	5,57
#3	12	1,13	5,50

9.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99.09 % (95% confidence interval: 95.04% - 99.98%).

9.3. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 99.25 % (95% confidence interval: 95.88% - 99.98%).

9.4. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

9.5 Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- *This kit is intended for research use only. All information and results associated with this kit is to be used for research use only.*
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- The NeoBiotech ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

11.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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SCHEME OF THE ASSAY

Cytomegalovirus (CMV) IgG

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut-off control	Sample (diluted 1+100)
Negative control	-	100µl	-	-	-
Positive control	-	-	100µl	-	-
Cut-off control	-	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well three times with 300µl of washing solution					
Conjugate	-	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution					
TMB Substrate	100µl	100µl	100µl	100µl	100µl
Incubate for exactly 15 min at room temperature in the dark					
Stop Solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					