DRG® CMV IgG ELISA (EIA-5153)

RUO

Revised 15 Sept. 2010 rm (Vers. 1.1)

TEST PRINCIPLE

The **DRG CYTOMEGALOVIRUS IgG ELISA** is a microtiter strip enzyme immunoassay for the detection of IgG class antibodies to Cytomegalovirus in human serum. This kit is intended for Research Use Only.

Microtiter strip wells as a solid phase are coated with Cytomegalovirus antigens. **Diluted** donor specimens and **ready-for-use** controls are pipetted into these wells. During incubation Cytomegalovirus-specific antibodies of positive specimens and controls are bound to the immobilized antigens.

After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes.

After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color is turned into yellow by stopping the enzymatic indicator reaction with sulfuric acid.

The intensity of this color is directly proportional to the amount of Cytomegalovirus-specific IgG antibody in the donor specimen.
Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Total incubation time: **1 hour, 45 minutes.**

Reagents and contents

12 **Cytomegalovirus microtiter strips (IgG)**
8-well snap-off strips coated with Cytomegalovirus antigens;
incl. 1 strip holder and 2 cover foils

100 ml **IgG sample diluent*****
Ready for use, colored **yellow**; pH 7.2 ± 0.2

14 ml **Stopping solution**
Ready-for-use sulfuric acid, 0.5 mol/l H_2SO_4

Warning
Sulfuric 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!

2 x 30 ml **Washing solution***
20x concentrated for 600 ml; pH 7.2 ± 0.2

20 ml **Cytomegalovirus anti-IgG conjugate****
Ready for use, colored red, **colorless** cap;
rabbit anti-human IgG, peroxidase conjugated

14 ml **TMB solution**
Ready-for-use 3,3',5,5'-tetra-methyl-benzidine

2 ml **Cytomegalovirus IgG positive control*****
Ready for use, colored yellow,**red** cap

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2 ml **Cytomegalovirus IgG negative control*****
Ready for use, colored yellow, **yellow** cap

***⇒Contains 0,3 % ProClin 300**

****⇒Contains 0,3 % ProClin 300 + 0,01 % Gentamycinsulfat**

*****⇒Contains 0,3 % ProClin 300 + 0,1 % Kathon**

Warning

All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies and HBsAg with 3rd-generation assays and have been found to be non-reactive.

The Cytomegalovirus IgG positive control has been found to be non-infectious in cell cultures.

Nevertheless, all materials should still be regarded and handled as potentially infectious.

Stability and storage

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

Do not use reagents after expiry date !

Reagent preparation

Working washing solution

Dilute *washing solution* **1+19** (e.g. 10 ml + 190 ml) with **fresh and germ free redistilled** water; consumption: **~5 ml** per determination

Crystals in the solution disappear by warming up to 37 °C in a water bath.
Stability after dilution: 4 weeks at 2 to 8 °C

Microtiter strips

The strips are vacuum sealed. Immediately after removal of strips, the remaining strips should be resealed in the plastic bag **along with the desiccant** supplied in the kit and stored at 2 to 8 °C.

Important notes

- **This test kit has been produced 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 donor samples and dispense conjugate without splashing accurately to the bottom of wells.**
- **During incubation cover microtiter strips with foil to avoid evaporation.**

Sample preparation

Dilute each donor specimen **1+100** with ***IgG sample diluent***;
e.g. 10 µl of specimen + 1 ml of IgG sample diluent (mix well).

Please note: *Positive and negative controls* are ready for use and must not be diluted!

TEST PREPARATIONS

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described.

It is very important to bring all reagents, samples and controls to room temperature before starting the test run!

Prior to commencing the assay, the **distribution and identification plan** for all specimens and controls should be carefully established on a form supplied in the kit.

Select the required number of *microtiter strips or wells* and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the *substrate blank,*
2 wells (e.g. B1+C1) for the *negative control* and
1 well (e.g. D1) for the *positive control*.

It is left to the user to determine controls and donor samples in duplicate, if necessary.

Test procedure

Step 1

a) Pipette

100 µl of **ready-for-use *negative control*** into wells B1 + C1,
100 µl of **ready-for-use *positive control*** into well D1 and
100 µl of **each d i l u t e d *donor sample***
into remaining wells corresponding to distribution plan.
Leave well A1 for substrate blank!
Cover wells with foil supplied in the kit.

b) Incubate for
1 hour at 37 °C.
During incubation time prepare the required volume of *working washing solution* sufficient for the number of wells used. (see "Reagent preparation").

c) Aspirate off contents of wells and wash them **3 times** with
300 µl of ***working washing solution.***
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Step 2

a) Dispense
100 µl of **ready-for-use *Cytomegalovirus anti-IgG conjugate*** into all wells **except A1** and cover them with foil.

b) Incubate for
30 minutes at room temperature (20 to 25 °C).
Do not expose to direct sun light!

c) **Repeat washing procedure** as described in step 1 c).
Note: Carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Step 3

a) Dispense
100 µl of **ready-for-use *TMB solution* into all** wells and cover them again.

b) Incubate for **exactly**
15 minutes at room temperature (20 to 25 °C) in the dark.

c) Dispense
100 µl of ***stopping solution*** (like **TMB**) into **all** wells.
Any blue color developed during the incubation turns into yellow.

Note: Highly positive donor samples can cause dark precipitates of the chromogen!

MEASUREMENT

Adjust the ELISA microplate or microstrip 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 donor 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.

Validation of the test run

The test run may be **considered valid**
provided the following criteria are met:

- ***Substrate blank*** **in A1:** ⇒ Absorbance value **lower than 0.100.**
- ***Negative control*** **in B1** and **C1:**
  ⇒ Absorbance value **lower than 0.300.**
- ***Positive control*** **in D1** should have reached an absorbance value **equal to** or **greater than** the **"Cut-off value".**

Results

Mean absorbance value of negative control [MN]

Calculate the mean absorbance value of the 2 negative control determinations (e.g. in B1/C1).
Example: (0.080 + 0.090) ÷ 2 = 0.085 = MN

Cut-off (value) [CO]

Add **0.250** to **MN.**

CO = MN + 0.250

Example: CO = MN + 0.250 = 0.085 + 0.250 = 0.335

Donor (mean) absorbance values
more than 10 % above CO
⇒ **POSITIVE**

Donor (mean) absorbance values
from 10 % above to 10 % below CO
⇒ **grey zone** ⇒ **repeat test**

2 - 4 weeks later - with <u>new</u> donor samples

Results *in the second test* again in the grey zone
⇒ **NEGATIVE**

Donor (mean) absorbance values
more than 10 % below CO
⇒ **NEGATIVE**

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Results in DRG UNITS [DU]

$$\frac{\text{Donor (mean) absorbance value} \times 10}{\text{CO}} = \text{[DRG UNITS = DU]}$$

Example: $\frac{1.580 \times 10}{0.335} = 47$ DU (DRG UNITS)

Interpretation of results

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

Sensitivity / Specificity

Sensitivity: 98%
Specificity: 100%