VIROTECH CMV IgG/IgM ELISA
(CMV IgG/IgM ELISA)

Order No.: EC113.00

Including performance data for CSF diagnostics

Colour code: yellow/transparent

FOR IN VITRO DIAGNOSTICS ONLY

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Contents

1 Intended Use

The VIROTECH CMV ELISA is used for the semiquantitative and qualitative detection of IgG and IgM antibodies against cytomegalovirus (CMV) in human serum or plasma (EDTA, Heparin, CPD or Citrate). It can also be used to perform parallel tests of serum-CSF pairs for the quantitative detection of endogenous CNS synthesis of IgG antibodies.

2 Diagnostic Relevance

Epidemiology and incidence

CMV is an ubiquitously distributed virus. The rate of infection is dependant on the socio-economic status of the patient examined. It is about 50% in industrial countries, while in developing countries it is almost 100%. The routes of transmission are droplet and contact, intimate intercourse, transfusions, transplantations and prenatal infections. An infection with CMV can occur in three different stages: as primary infection, as latency or as reactivation (1, 2, 3).

As with other herpes viruses the primary infection is followed by life-long latency, when no symptoms occur. Latency is defined as a form of reversible, nonproductive infection of the host by virus capable of reproduction. Reactivation can be brought about by a powerful renewed multiplication of the virus or by renewed infection. A CMV infection usually progresses subclinically in healthy persons. The symptoms of a CMV infection are those of a viral syndrome. The symptoms are fever, fatigue, sore throat, heterophilic lymphocytosis and liver malfunction. In addition there can also be direct organ damage such as pneumonia, retinitis, colitis/oesophagitis, hepatitis, mononukleosis and meningoencephalitis (2, 3, 5).

Pregnant women:

The most severe effects are those on neonates who have been infected *in utero*. This occurs mainly as a result of primary infection of the woman during pregnancy. Such a congenital infection can lead to severe consequences for the neonate, such as severe mental damage, deafness or death.

CMV is the most frequent infection of the newborn. The disease itself breaks out in appreciably less than 10% of infected neonates. The outbreak of the disease in the foetus or neonate is dependent on many variables that have not yet been studied (3, 4, 6).

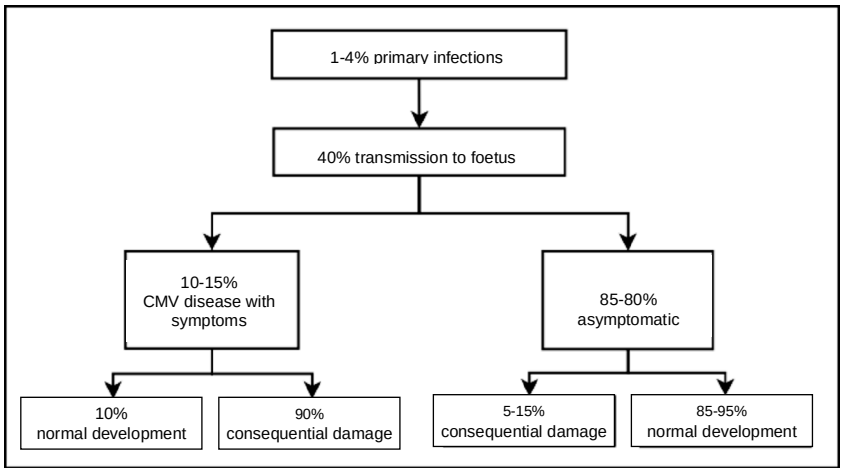


Figure 1 Proportion of primary reactions in all pregnancy and proportion of subsequent damage

Transplant recipients and immunosuppressed patients:

The immune system of transplant recipients is suppressed in order to avoid rejection reactions against the transplanted organ. This becomes critical if the donor is CMV positive and the recipient CMV negative. CMV infections precipitate rejection reactions in organ tranplantation.

The second group is made up of patients with insufficiency of the immune system, such as occurs for example after HIV infection. Here too the immune system is too weak to prevent the outbreak of a disease caused by CMV. Typical damage such as retinitis occurs in this group of patients.

When there is reactivation an IgM respose is not to be expected from immunocompetent patients, but rather in the immunosuppressed (2, 3).

3 Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4 Package Contents (IgG/IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3ô5,5ôTMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5 Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in the dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
PBS-Dilution Buffer	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6 Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7 Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator
11. VIROTECH RF-SorboTech (order number: 161101, 161102 or B/300.00)

8 Test Procedure SERUM DIAGNOSTICS

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma (EDTA, Heparin, CPD or Citrate) can be used as test material, even if only serum is mentioned in the instructions. Comparative data for serum and plasma is available on request.

Always prepare dilution of patient sample freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready-to-use controls (positive control, negative control, cut-off control) are parameter specific and only to be used with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG and IgM-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off

control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.

2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 ï 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9 Test Evaluation SERUM DIAGNOSTICS

The ready to use controls serve for a semiquantitative determination of specific IgG and IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE_{(positive\ control)} = \frac{OD_{(positive\ control)}}{OD_{(cut\text{-}off\ control)}} \times 10$$

$$VE_{(patient\ serum)} = \frac{OD_{(patient\ serum)}}{OD_{(cut\text{-}off\ control)}} \times 10$$

9.3 Interpretation Scheme IgG and IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 ï 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results should always include the clinical picture, epidemiological data and any other laboratory findings that are available.
2. The ELISA is not designed to diagnose a CMV infection with risk patients suspected of acute infection. A direct detection procedure is to be preferred for immune compromised patients and pregnant women. Neonates with congenital CMV infections can be serologically normal, so that virus isolation must be attempted within the first two weeks of life if there is a suspicion of infection.
3. The cross reaction between CMV and other Herpes viruses can yield a false positive result. This is caused by polyclonal stimulation of B lymphocytes, cross reactivity between other Herpes viruses such as EBV or HHV 6 must always be expected. Furthermore the possibility of cross reactions between CMV and Parvovirus cannot be excluded.
4. In order to reduce the risk very different types of differential diagnosis are recommended depending on the clinical situation and the symptoms presented, in retinitis of the HIV infected for example toxoplasmosis, in mononucleosis of immunocompetent patients for example Epstein-Barr virus

10 Performance Data SERUM DIAGNOSTICS

10.1 Sensitivity and specificity

Sensitivity and specificity were determined by testing 569 sera in VIROTECH CMV IgG ELISA and 672 sera in VIROTECH CMV IgM ELISA in comparison with a reference ELISA.

IgG sensitivity and specificity

Serum collection (n=569)

VIROTECH CMV IgG ELISA	Reference ELISA		
	negative	**borderline**	**positive**
negative	186	0	4
borderline	0	0	6
positive	1	1	371

This yields a sensitivity of 98.9% and a specificity of 99.5% for IgG.

IgM sensitivity and specificity
Serum collection (n=672)

VIROTECH CMV IgM ELISA	Reference ELISA		
	negative	**borderline**	**negative**
negative	327	10	11
borderline	4	4	5
positive	5	5	301

This yields a sensitivity of 96.5% and a specificity of 98.5% for IgM.
Borderline sera were not included in the calculation.

Diagnostic sensitivity
The diagnostic sensitivity was determined by testing 81 clinically characterized sera in VIROTECH CMV IgG ELISA and 83 in VIROTECH CMV IgM ELISA.

IgG diagnostic sensitivity
Serum collection (reactivations, n=81)

VIROTECH CMV ELISA	negative	borderline	positive
	2	0	79

This yields a sensitivity of 97.5%

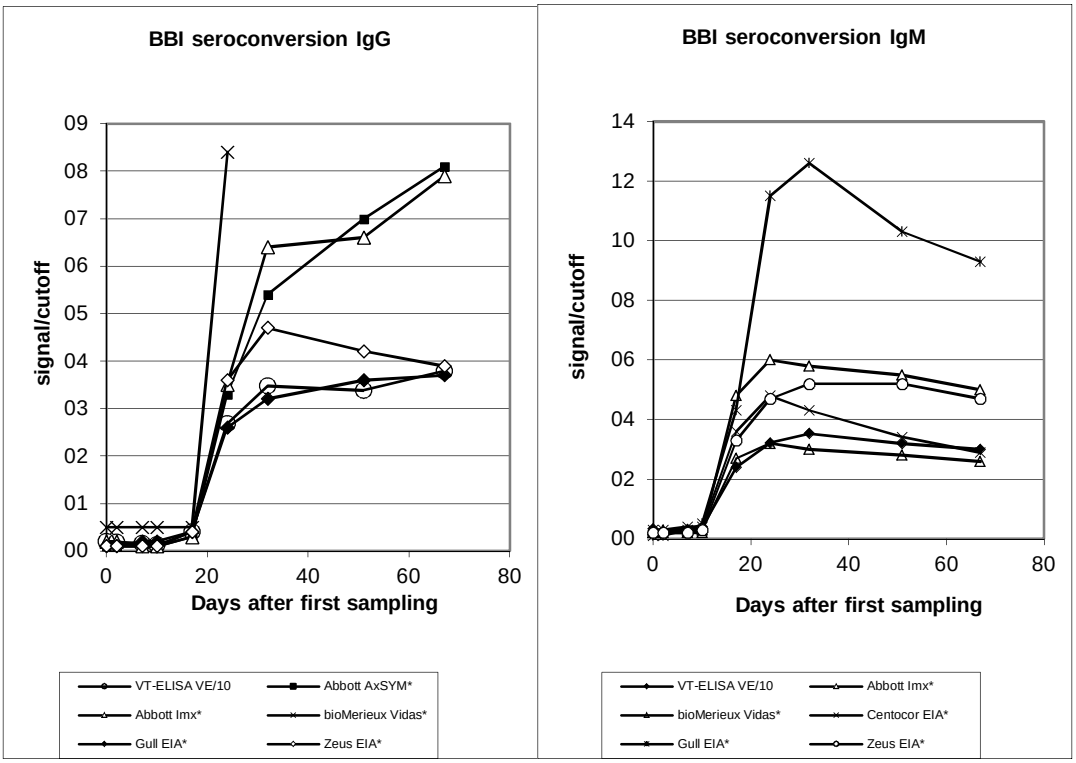
IgM diagnostic sensitivity
Serum collective (reactivations, n=80 and primary infections, n=3)

VIROTECH CMV ELISA	negative	borderline	positive
	5	1	77

This yields a sensitivity of 93.9%.
Of the 5 negative sera one serum also tested negative and one serum as borderline in the reference test.

10.2 Detection limits

The seroconversion panel (PTC 901) was tested. The VIROTECH CMV ELISA exhibited the expected sensitivity in IgG and in IgM.



* Original data from BBI

The bioMerieux Vidas exhibited values >9.0 for signal/cut off in the IgG values for the last three samples taken.

10.3 Cross reactivity

The following sera were tested in IgG to determine cross reactivity.

Pathogen	Number tested,	neg	bor	pos
VZV	35	14	2	19
HSV1	12	4	1	7
HSV2	20	4	0	16
Measles	37	15	1	21
EBV	39	16	2	21
Parvovirus	20	11	0	9

All positive results were confirmed in the reference test. Only one serum exhibited a false positive result. This was an EBV positive serum.

The following sera were tested in IgM to determine the cross reactivity.

Pathogen	Number tested	neg	bl	pos
EBV	23	17	1	5
VZV	22	21	0	1
Parvovirus	20	17	1	2
Measles	13	12	1	0
HIV	20	14	2	4

The positive results also reacted as positive in a reference test.

10.4 Intraï assay coefficient of variation (repeatability) IgG and IgM

The intra-assay coefficient of variation was determined by using 12 strips from various plates of a batch in a test series. All 96 wells were tested with one serum.

	IgG CV %	IgM CV %
positive	8.2	4.2
borderline	9.0	5.7
negative	10.2	5.6

10.5 Inter assay coefficient of variation (reproducibility)

In the case of IgG 12 different test series were carried out in different laboratories and by different testers with 7 positive sera, 1 borderline and one negative borderline serum.

IgG inter-assay coefficient of variation

Serum	Mean of VE value	CV%
borderline	10.0	9.4
negative-borderline	9.0	9.1
positive	26.8	10.0
positive	38.4	8.8
positive	25.0	8.5
positive	28.0	10.8
positive	27.4	12.1
positive	27.3	14.1
positive	42.9	11.6

In the case of IgM 10 different test series were carried out in different laboratories and by different testers with 5 positive sera, 1 borderline-negative and 3 negative sera.

IgM inter-assay coefficient of variation coefficient

Serum	Mean of VE value	CV%
negative	5.0	3.4
negative	1.7	10.7
negative	2.2	13.1
negative borderline	8.5	6.9
positive	12.4	3.5
positive	14.3	4.1
positive	44.3	4.2
positive	31.0	4.3
positive	19.6	2.8

11 Performance Data CSF DIAGNOSTICS TESTING

11.1 Sensitivity

To determine the sensitivity of the CMV CSF IgG ELISA, 25 CSF/serum pairs were compared with a reference ELISA.

CSF-sera samples (n=25)

VIROTECH CMV CSF IgG ELISA	Reference ELISA	
	normal	pathological
normal	0	0
pathological	1	24

This gives a sensitivity of >99.9%.

The false positive is a pathological serum/CSF pair, which was not recognized in the reference test.

11.2 Specificity

To determine the specificity of the VIROTECH CMV CSF IgG ELISA, 26 CSF/serum pairs were compared with a reference ELISA.

CSF-sera group (n=26)

VIROTECH CMV CSF IgG ELISA	Reference ELISA	
	normal	pathological
normal	24	1
pathological	0	1

This gives a specificity of >99.9%.

11.3 Intra-assay coefficient of variation (repeatability)

To determine the intra-assay coefficient of variation, a CSF/serum pair with normal AI value and a CSF/serum pair with pathological AI were tested 10 times in a run.

	CV%
Normal AI	12.2
Pathological AI	7.5

11.4 Inter-assay coefficient of variation (reproducibility)

To determine the inter-assay coefficient of variation, a CSF/serum pair with normal AI value was tested 10 times in different laboratories by different workers. A CSF/serum pair with normal AI value with pathological AI value was tested 11 times in different laboratories by different workers.

	CV%
Normal AI	16.0
Pathological AI	8.5

12 Literature

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6. Lazzarotto, T., L. Gabrielli, M. Lanari, B. Guerra, T. Bellucci, M. Sassi, and M. P. Landini. 2004. Congenital cytomegalovirus infection: recent advances in the diagnosis of maternal infection. Hum Immunol 65:4105.

Preparation of Patient Samples and Washing Solution

- **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

- **IgG-Samples - Dilution 1:101**

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

- **IgM-Samples - Dilution 1:101**
Rheumafactor-absorption with RF-SorboTech

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇓		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇓		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgM, IgA
⇓		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇓		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇓		
Stopping		**50 µl Stopping Solution** shake carefully
⇓		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**