

## ELISA ANTI - MYCOPLASMA IgG/ IgM/ IgA

### 1. INTENDED USE

Enzyme-immunoassay for determination of antibodies against Mycoplasma pneumoniae in human serum and plasma

IgG [REF] VEG 114 s 96 [IVD]  
IgM [REF] VEM 114 s 96 [IVD]  
IgA [REF] VEA 114 s 96 [IVD]

### 2. PRINCIPLE OF THE ASSAY

The detection of antibodies is based on the principle of an enzyme-linked immunosorbent assay (ELISA). The purified, homogeneous antigen is fixed to each well of the microtiterstrips. Any specific antibodies present in the patient's sample are bound during the first incubation. After removing unbound material by washing, the presence of specific antibodies is detected using anti-human IgG/ IgM/ IgA conjugate during the second incubation. Excess peroxidase conjugate is then removed and TMB substrate is added, resulting in the development of a blue colour. The enzyme reaction is terminated by the addition of a stop solution. The intensity of the yellow colour thus developed is proportional to the concentration of antibodies in the sample.

### 3. DIAGNOSTIC RELEVANCE AND INTERPRETATION OF RESULTS

To obtain a final diagnosis the patient history and clinical symptoms should be included for the interpretation of the serological results and possible cross-reactivity should be taken into consideration.

IgG	IgM/IgA	Interpretation	Recommendation
-	-	no specific antibodies detectable	<b>Monitoring</b> where an acute infection is suspected (Sample collection within 10-14 days).
+	-	probable previous infection	<b>Monitoring</b> of IgG antibodies (Sample collection within 10-14 days), a significant titre increase in absence of IgM antibodies indicates reinfection
-	+	probable first stage of a primary infection	<b>Monitoring</b> of IgG, IgM and IgA antibodies (Sample collection within 10-14 days) to determine seroconversion; confirmatory tests e.g. IFA, CF
+	+	acute infection probable; reinfection possible	<b>Monitoring</b> of IgG, IgM and IgA antibody titres; confirmatory tests e.g. IFA, CF
+	-	reinfection probable,	<b>Monitoring</b> ; significant IgG and IgA antibody titre increase. Sample collection at an interval of 5-10 days. Confirmatory tests e.g. IFA, CF

- negative, + positive

### 4. PERFORMANCE CHARACTERISTICS

#### Specificity / Sensitivity

51 samples IgG, 64 samples IgM, IgA were tested parallel in ELISA ANTI-MYCOPLASMA IgG/IgM/IgA and comparison methods (ELISA). The sensitivity and specificity are based on the results found.

**Specificity:** IgG 94%      **Sensitivity:** IgG 88%  
**Specificity:** IgM/IgA 95%      **Sensitivity:** IgM/IgA 100%

For the calculation of the sensitivity and specificity of the ELISA test, equivocal results were defined as positive results. The results refer to the groups of samples investigated.

#### Precision and reproducibility

Intra-assay reproducibility was determined by testing samples of different levels of antibody reactivity for at least 22 times in one test run. The coefficient of variation (CV) of the reactive IgG, IgM and IgA samples was < 10%.

Inter-assay reproducibility was determined by testing samples of different levels of antibody reactivity in 10 different test runs. The CV of the reactive IgG and IgM samples was < 10%.

### Cross-reactivity

The detection of antibodies against Mycoplasma pn. in Particle Agglutination test (HAT) with Glycolipid extraction of the antigen is not agent specific (false positive reaction). The Mycoplasma pn. antigen used in ELISA is an extracted, purified (N<sub>2</sub> washed) complete antigen, so that the antigen is multiple specific. Cross reactions are not really excluded (respiratory antibodies). With acute Mycoplasma hominis is a false positive reaction in Mycoplasma pn. IgM tests as well as in IgM tests of the herpes group possible. The homologous IgM antibodies are more developed than the heterologous IgM antibodies, false positive reactions are rare.

### 5. [Bib]

- Hahn, H.; Falke, D.; Klein, P.: Med. Mikrobiol. (1991) Springer-Verlag  
- Jacobs, E.; Klin. Lab. 40, 228-229 (1994)

### 6. KIT COMPONENTS

- MICROTITERSTRIPS** [MTS]  
One microtiterplate is supplied which contains 12 microtiterstrips of 8 breakapart wells. The wells are coated with purified, inactive antigen. Strips are colour-coded.
- PEROXIDASE CONJUGATE** [CONJ|POD]  
One vial containing 12 ml of ready-to-use anti-human IgA, IgG or IgM Peroxidase Conjugate. Peroxidase conjugate contains 0.049% Thimerosal as preservative.
- NEGATIVE CONTROL** [CONTROL|-]  
One vial of 1.2 ml containing human serum with 0,095% sodium azide as preservative. Ready to use.
- CUT OFF CONTROL** [CUTOFF]  
One vial of 1.2 ml containing human serum with 0,095% sodium azide as preservative. Ready to use.
- POSITIVE CONTROL** [CONTROL|+]  
One vial of 1.2 ml containing human serum with 0,095% sodium azide as preservative. Ready to use. The titre is reported on the label. Is no titre indicated on the label, the [CONTROL|+] is a positive reference sample and has a ratio  $\geq 1,500$ .
- TMB SUBSTRATE** [SUBS|TMB]  
One vial containing 13 ml of ready-to-use tetra-methylbenzidine (TMB) substrate.
- SAMPLE DILUENT** [SPE|DIL]  
One bottle containing 100 ml (2x50 ml) of ready-to-use sample diluent buffer. The buffer includes 0.049% Thimerosal as preservative.
- WASH SOLUTION 25X** [WASH|BUF|25x]  
One bottle containing 80 ml (2x40 ml) of wash solution concentrate.
- STOP SOLUTION** [SOLN|STOP]  
One bottle containing 15 ml of 0.95N H<sub>2</sub>SO<sub>4</sub> stop solution. Ready-to-use.

*The safety data sheet (MSDS) is available upon request.*

### 7. STORAGE AND STABILITY

Store all reagents at 2-8°C. Protect them from intense light and do not freeze. The expiration date of each component is indicated on the respective vial label. Do not use reagents beyond the expiration date. After opening, [MTS] must be stored at 2-8°C in the plastic bag with desiccant and are stable up to 4 weeks. The diluted [WASHBUF] is stable up to 4 weeks when stored at 2-8°C. Use only [MTS] with an intact vacuum packaging.

### 8.

#### MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Test tubes for sample dilution
- Timer
- Micropipettes, multipipettes 10-1000 µl
- One-liter graduated cylinder, distilled water
- ELISA washer or multichannel pipette
- Spectrophotometer for micro-plates (450 nm/ reference wavelength 630/620 nm)
- Paper towels, pipette tips

## 9. WARNINGS OR PRECAUTIONS SAFETY PRECAUTIONS

The ELISA test is for [IVD] use only.

- Only qualified and well-trained employees should carry out the assay procedure.
- The instruction for use describes the applicable test method. In case of modification or applications others than the intended use, or the use of automatic processors, the user has to validate the procedure and take the responsibility for it.
- Do not mix lot specific reagents, such as [MTS], controls and [CONJ]POD from different kit lots. The [SUBS]TMB does not have to be from the original test kit, but the lot of the [SUBS]TMB has to be the same as indicated on the kit label. The [SPE]DIL (except Immunocapture assays), the [WASHBUF]25x and the [SOLN]STOP can be used for all ELISA tests.
- Seal all bottles properly after use in order to avoid bacterial contamination. All samples and kit components should be considered potentially infectious. All control samples have been tested for Hepatitis Bs antigen, anti-HIV I and II, anti-HCV (CE/FDA) and found to be negative.
- The [MTS] are coated with inactive antigen. However, normal laboratory precautions should be maintained when handling with infectious material. Do not pipette by mouth.
- Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see kit contents). Wash thoroughly with water in case of contact and possibly look up a doctor.
- Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.
- The [SOLN]STOP (0,95 N H<sub>2</sub>SO<sub>4</sub>) contains sodium hydroxide which may irritate skin and mucous membranes. Wash thoroughly with water in case of contact.
- For disposal the legal regulations have to be followed.

## 10. SPECIMEN COLLECTION AND STORAGE

- Microbially contaminated specimen may cause interference.
- Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
- Suitable specimens are serum or plasma (heparinized, EDTA) samples obtained by standard laboratory techniques.
- The samples should not be heat-inactivated since non-specific results may occur. Avoid repeated freeze-thaw cycles.
- Results on tests using CSF are not available.
- Patient samples should be stored at t2-8°C. For long term storage t-20°C or lower is recommended. Avoid repeated freeze-thaw cycles.
- Note:** Diluted patient samples must be used on the same day.

## 11. ASSAY PROCEDURE REAGENT PREPARATION

Bring all reagents to room temperature prior to use !

[WASHBUF] : Dilute the [WASHBUF]25x 1:25 with distilled water e.g. add 40ml of [WASHBUF]25x to 960 ml distilled water and mix well.

**Dilution of samples:** Dilute patient samples 1:101 with [SPE]DIL e.g. 10µl sample + 1ml [SPE]DIL; mix thoroughly.

In case of pretreatment of the samples with IgG-RF-Sorbent, the final dilution of 1:101 must be considered. Please follow the instruction for use of the IgG-RF-Sorbent used in the test.

**Controls are ready to use.**

**Note:** To take into consideration pipetting time, it is recommended that the [CUTOFF] is repeated after every 4 [MTS] (resp. after a pipetting time of >=5 min.) to evaluate the following patients tests with the new calculated cut-off value. In case of a semiquantitative determination the [CONTROL+] should also be repeated the same way the [CUTOFF] was dispensed.

Take the required [MTS] out of the foil packets and place them in the holder. Possibly remaining wells of a [MTS] have to be stored at 2-8°C tightly sealed in the plastic bag provided, with the desiccant inside.

## 12. PIPETTING AND INCUBATION STEPS

- Pipette 100µl of the controls or diluted patient sample into the wells. Pipette 100 µl of sample diluent into well A1 (Blank).
- Incubate the wells at room temperature (21-25°C) for 30 minutes, protected from intense light.
- Wash the wells four times as described in section k. WASHING PROCEDURE
- Add 100µl of ready-to-use peroxidase conjugate to each well.
- Incubate the wells at room temperature (21-25°C) for 30 minutes, protected from intense light.
- Repeat washing as in section C above.
- Add 100µl of ready-to-use TMB substrate to each well.
- Incubate the wells at room temperature (21-25°C), in the dark for 10 minutes
- Add 100µl of stop solution to each well. Tap gently to ensure homogenous color distribution and read within 10 minutes.
- To read the plate, make sure that the bottom is free from moisture and that no air bubbles are in the wells. Read the absorbance of the well contents at 450nm on a suitable plate reader. On readers equipped with a dual wavelength facility set the reference filter to 620/630 nm.

**Attention:** The absorbance (OD) of the Blank must be always subtracted from the OD values of the controls and samples.

## PROCEDURAL NOTES

Do not allow the wells to dry out between incubations. Comply with the given incubation temperatures and times.

## k. WASHING PROCEDURE

The washing procedure can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel. Wash four times with a soaking time of approx. 30 seconds (300 µl ).

## 13. SUGGESTIONS FOR TROUBLESHOOTING

In case that the ELISA instructions are followed strictly, the reagents are handled with care and the samples and reagents are pipetted carefully, the following kinds of errors can be avoided to a large extent.

ERROR	POSSIBLE CAUSES
No colourimetric reaction after addition TMB substrate	No Peroxidase conjugate pipetted, contamination of Peroxidase conjugate (possibly with control sera during pipetting) may cause an inactivation.
Generally too high reaction	Incorrect Peroxidase conjugate (i.e. not from original test kit), incubation time too long or incubation temperature too high, water quality for Washing Solution insufficient (low grade of deionization)
Generally too weak reaction	Incorrect Peroxidase conjugate (i.e. not from original test kit), incubation time too short, incubation temperature too low
Reagent blank too high	Incorrect pipetting of sample diluent, contaminated reagents, reagents expired, exceeding of incubation time and temperature, external contamination of the bottom of microtiterstrips. (clean carefully!)
False positive / negative samples	Incorrect dilution of samples, microbially contaminated specimen
Unexplainable outliers	Contamination of pipettes, tips or containers or with metals (iron, copper etc.), insufficient washing
High variation (within a series)	Reagents (including microtiterstrips) not pre-warmed to room temperature prior to use. Washer is not washing correctly!
High variation (from series to series)	Incubation conditions not constant (time, temperature) high variation of incubation temperature, controls and samples are not carried out at same time (same intervals) check pipetting order, person related variation, strips dried out after washing (unreproducible results)

## 14. VALIDITY OF THE ASSAY

All controls should be carried out with every test run.

The test must comply with the following validation criteria:

- OD-value of Negative Control should be  $< 0.100$ ,
- OD-value of Cut-off Control should be  $> 0.200$ ,
- Ratio of Positive Control/ Cut-off value should be  $\geq 1.5$
- OD-value of the Blank IgA should not be higher than 0,100.
- OD-value of the Blank IgG, IgM should not be higher than 0,200.

If controls give invalid levels then results from the test samples are invalid too and retesting is required.

## 15. CALCULATION OF RESULTS

### A QUALITATIVE CALCULATION

#### Calculation of Cut-off Value

The **Cut-off Value** is calculated from the absorbance of the Negative Control and the absorbance of the Cut-off Control and defines the Cut-off range.

$$\text{Cut-off Value} = \text{OD of the Negative Control} + \text{OD of the Cut-off Control}$$

$$\text{CUT-OFF RANGE} = \text{CUT-OFF VALUE} \pm 10\%$$

Interpretation of sample results:

RESULT	DEFINITION
<b>negative -</b>	OD value sample $<$ Cut-off value <b>-10%</b>
<b>equivocal</b>	OD value sample $\geq$ Cut-off value <b>-10%</b> OD value sample $\leq$ Cut-off value <b>+10%</b>
<b>positive +</b>	OD value sample $>$ Cut-off value <b>+10%</b>

Equivocal results should be retested. Following the confirmation of the equivocal result the monitoring of the patient's antibodies is recommended in order to exclude unspecific reactions resp. cross-reactivity, which may also cause equivocal results.

**Note for IgM assay:** In case of a positive or equivocal IgM antibody detection these samples should be confirmed with ALPHADIA ELISA IgG RF-Sorbent [REF] VSB 100.

### B CALCULATION OF RATIO (CUTOFF INDEX, COI):

Patient samples may also be quantified and interpreted by means of the calculation of the ratio (Cutoff Index, COI):

$$\text{COI} = \text{OD value of sample} / \text{Cut-off value},$$

whereby a ratio of 1.000 is equivalent to the Cut-off value.

Interpretation of sample results:

- Ratio  $< 0,9$**  negative result
- Ratio  $0,9-1,1$**  equivocal result
- Ratio  $> 1,1$**  positive result

### C SEMI-QUANTITATIVE TITRE CALCULATION

A semi-quantitative diagram is enclosed. The first point on the curve is obtained from the **Cut-off value** (y-axis) and the cut-off titre 1:100 (x-axis). The second point of the curve is obtained from the absorbance of the [CONTROL+] (y-axis) and their titre (x-axis) as indicated on the label. Drawing a straight line between the two points produces the semi-quantitative curve. The titre of the patient samples may be read from the curve. The graph is linear up to the titre of the [CONTROL+].

Samples with titres higher than the titre of the [CONTROL+], should be diluted further with [SPE]DIL according to the expected titre. For calculation of results, the dilution factor should be taken into consideration.

The calculated titres of the patient samples may also be indicated as VU (VIRO-Units), e.g. a titre of 1:250 is equivalent to 250 VU.

The [CONTROL+] for ANTI-MYCOPLASMA IgA is a *positive reference sample* with a ratio  $\geq 1,500$ . A quantification of the test result by means of titre specifications is therefore not possible.

Regarding diagnostic relevance and interpretation of results see **page 1**.

## Símbolos/ Simboli/ Simbolos/ Symboly/ Címkékén/

## IVD

[MTS]	Mikrotiterstreifen/ microtiterstrips/ Microplaques sensibilisées/ placa de microtítulo/ piasta microtítulo/ Tiras de microtitulação/ Mikrotitra ní stripy/ Mikrotitercskok/
[SPE]DIL	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ soluzione tampone/ estabilizador de diluição/ edici roztok vzorku/ Mintahígító/
[WASHBUF]25x	Waschlösung/ wash solution/ solution de lavage/ solución limpiadora/ soluzione lavaggio/ solução de lavagem Konzentrat/ concentrate / Concentré / concentrado/ concentrato/ concentrado 25x/ Promývaci roztok 25x/ Mosópufferkoncentrátum 25x/ $\mu$ 25x
[CONJ]POD	Peroxidase-Konjugat / Peroxidase conjugate / Conjugue Peroxidase/Conjugado Peroxidasa / coniugato con perossidasi/ conjugado Peroxidase/ Peroxidázový konjugát / Peroxidáz Konjugátum/
[CONTROL]-	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo / Negatívni kontrola/ Negatív Kontroll/
[CUT_OFF]	Cut-off Kontrolle/ cut-off control/ Contrôle cut-off/ control valor límite/ controllo limitante/ controle interrupção/ Cut off kontrola/ cut off kontroll/
[CONTROL+]	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo/ Pozitivní kontrola/ pozitív kontroll/
[SUBS]TMB	TMB-Substrat/ TMB substrate/ substrat TMB/ substrato TMB / TMB Substrát/TMB Szubsztrát/ $\mu$ TMB
[SOLN]STOP	Stopplösung/ stop solution/ Solution d'arrêt/ solución de parada/ soluzione d'arresto/ solução de parada/ Stop pufr/ Stop Oldat/ $\mu$ $\mu$ $\mu$
[Bib]	Literatur/ Literature/ Littérature/ Bibliografia/ Bibliografia/ Literatura/ Irodalom/ o
[LOT]	Charge/ lot/ Lot/ lote/ carcia/ lote/ íslo zar0e/ Lot Szám/ $\mu$
[IVD]	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostic in vitro/ diagnóstico in-vitro/ In-vitro diagnostic/ diagnóstico In-vitro/ In vitro diagnostikum/In Vitro Diagnosztikum/ <sup>a</sup> In vitro
[REF]	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda/ Katalogové íslo/ Katalógusban Szerepl Kód/
<b>S</b> 96	96 Bestimmungen/ tests/ test / determinazioni/ testes / Po et test / Vizsgálatok Száma/ $\mu$
<b>I</b>	Gebrauchsanweisung beachten/ consult instructions for use/ consulter le mode d'emploi/consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruções de uso/ P e t te si Návod k použití/ Olvassa el a Használati Utasítást/ <sup>a</sup>
<b>t</b>	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura/ Teplotní limity/ H mérsékleti Korlátozások/ $\mu$
<b>e</b>	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade/ Datum expirace/ Lejárati Id / $\mu$ $\mu$ ( ...)
<b>M</b>	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por/ Vgyőrcce/ Gyártó/ ...
9/2008	
<b>M</b> :	<b>ALPHADIA</b> , Avenue Vésale 26, B-1300 Wavre, BELGIUM Tel.: +32 10 24 26 49 Fax: +32 10 24 55 99 email: contact@alphadia.be

## Symbole nach IVD/ symbols used with IVD devices/

### Symbole / Símbolos / Simboli / Simbolos IVD

MTS	Mikrotiterstreifen/ microtiterstrips/ Microplaques sensibilisées/ placa de microtítulo/ piasta microtítola/ placa do microtitre
SPE[DIL]	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ solução tampone/ estabilizador de diluição
WASHBUF[25x]	Waschlösung/ wash solution/ solution de lavage/ solución limpiadora/ soluzione lavaggio/ solução de lavagem Konzentrat/ concentrate / Concentré / concentrada/ concentrato/ concentrado 25x
CONJ[POD]	Peroxidase-Konjugat / Peroxidase conjugate / Conjugue Peroxidase/Conjugado Peroxidasa / coniugato con perossidasi/ conjugado Peroxidase
SUBS[TMB]	TMB-Substrat/ TMB substrate/ substrat TMB/ substrato TMB
SOLN[STOP]	Stopplösung/ stop solution/ Solution d'arrêt/ solución de parada/ solução d'arresto/ solução de parada
CONTROL+	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo
CONTROL-	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo
CUTOFF	Cut-off Kontrolle/ cut-off control/ Contrôle cut-off/ control valor limite/ controllo limitante/ controle interrupção
CAL1	Calibrator/ calibrator/ calibreur/ calibrador/ calibrator/ calibrador 1
CAL2	Calibrator/ calibrator/ calibreur/ calibrador/ calibrator/ calibrador 2
CAL3	Calibrator/ calibrator/ calibreur/ calibrador/ calibrator/ calibrador 3
CAL4	Calibrator/ calibrator/ calibreur/ calibrador/ calibrator/ calibrador 4
LOT	Charge/ lot/ Lot/ lote/ carcia/ lote
IVD	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostic in vitro/ diagnóstico in-vitro/ In-vitro diagnostic/ diagnóstico In-vitro
REF	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda
RTU	gebrauchsfertig/ ready to use/ Prêt à l'emploi/ listo para su uso/ pronto per l'uso/ pronto para usar
▽ 96	96 Bestimmungen/ tests/ testés/ determinazioni/ testes
i	Gebrauchsanweisung beachten/ consult instructions for use/ consultar la mode d'emploi/ consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruções de uso
T	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura
D	Verfallsdatum/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade
M	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por

## D ELISA TESTDURCHFÜHRUNG

### Vorbereitung der Reagenzien

#### Probenverdünnung:

Alle Untersuchungsproben 1:101 verdünnen  
z.B. 10 µl Probe + 1 ml [SPE[DIL].

Bei der Vorbehandlung der Proben mit RF-Absorbent, muss die Endverdünnung von 1:101 berücksichtigt werden. Bitte die Gebrauchsanweisung des im Test eingesetzten RF-Sorbent beachten.

Die Kontrollen/ Calibratoren sind [RTU]!

Benötigte Anzahl [MTS] den Folienverpackungen entnehmen und in den Halterahmen einsetzen. [MTS] nur an den Enden anfassen. Nicht benötigte [MTS] in den mit Trockenmittel versehenen Plastikbeutel geben

100µL BLANK (A1),KONTROLLEN UND VERDÜNNTE PATIENTENPROBEN PIPETTIEREN

1. INKUBATION 30 MIN. T R.T.\*

1. WASCHSCHRITT

Nach 1. Inkubation 4 mal mit hergestellter [WASHBUF] waschen

100 µl [CONJ[POD] je Vertiefung pipettieren

2. INKUBATION 30 MIN. T R.T.\*

2. WASCHSCHRITT

Nach 2. Inkubation 4 mal mit hergestellter [WASHBUF] waschen

100 µl [SUBS[TMB] je Vertiefung pipettieren

3. INKUBATION 10 MIN. T R.T.\*

Nach exakt 10 min die Enzym/Substratreaktion mit 100 µl [SOLN[STOP] pro Vertiefung beenden. Platte vorsichtig mischen, bis die Farbreaktion homogen erscheint.

PHOTOMETRISCHE MESSUNG

Entstandene Farbreaktion innerhalb von 10 min. bei 450 nm /Referenzwellenlänge 630/620 nm photometrisch gegen Blank messen.

\* T R.T. Raumtemperatur bis maximal 25°C

## GB ELISA ASSAY PROCEDURE

### Prepare reagents

#### Dilution of samples:

Dilute patient samples 1:101  
e.g. 10 µl sample + 1 ml [SPE[DIL].

In case of pretreatment of the samples with RF Absorbent, the final dilution of 1:101 must be considered. Please follow the instruction for use of the RF Absorbent used in the test.

Controls/ calibrators are [RTU]!

Take required [MTS] out of the foil packet and place them in the holder. Do not touch the wells. Unrequired [MTS] should be put in the plastic bag with desiccant and closed carefully

PIPETTE 100µL BLANK (A1),CONTROLS AND DILUTED SAMPLES

FIRST INCUBATION 30 MIN. room T \*

FIRST WASH STEP

After incubation wash the wells 4 times with [WASHBUF]

Pipette 100 µl [CONJ[POD] each well

SECOND INCUBATION 30 MIN. room T \*

SECOND WASH STEP

After incubation wash the wells 4 times with [WASHBUF]

Pipette 100 µl [SUBS[TMB] each well

THIRD INCUBATION 10 MIN. room T \*

After exaktly 10 min stop reaction by adding 100 µl [SOLN[STOP] each well. Shake plate carefully until colour is homogenous.

PHOTOMETRIC READING

Measure all Optical Density values (OD) within 10 min. against the Blank, with a spectrophotometer at 450 nm / 630 or 620 nm.

\* room T up to maximally 25°C

## F TECHNIQUE ELISA

### Préparation des réactifs

#### Dilution des échantillons:

Diluer les échantillons au 1:101  
10 µl échantillon + 1 ml [SPE]DIL.

En cas de prétraitement des échantillons avec le sorbent RF, il faut tenir compte d'une dilution finale de 1:101 des échantillons. Suivre attentivement les instructions d'utilisation du sorbent RF.

Les contrôles/calibrateurs sont [RTU]!

Enlever le nombre requis de [MTS] du sachet et les placer dans le cadre. Ne pas toucher les puits. Remettre les autres [MTS] dans le sachet avec le dessicant et fermer soigneusement

DÉPOSER 100µL DE BLANC (A1), DE CONTRÔLES ET DE CHAQUE DILUTION

PREMIÈRE INCUBATION 30 MIN. À .i. t.a.\*

PREMIER LAVAGE

Laver 4 fois les puits avec  
[WASH]BUF

Déposer 100 µl de [CONJ]POD dans chaque puits

DEUXIÈME INCUBATION 30 MIN. À .i. t.a.\*

DEUXIÈME LAVAGE

Laver 4 fois les puits avec  
[WASH]BUF

Déposer 100 µl de [SUBS]TMB dans chaque puits

TROISIÈME INCUBATION 10 MIN. À .i. t.a.\*

Après 10 min. précises, arrêter la réaction en ajoutant, à chaque puits, 100 µl de [SOLN]STOP  
Agiter le cadre jusqu'à l'homogénéisation de la coloration

LECTURE PHOTOMÉTRIQUE

Les densités optiques seront mesurées après 10 min., contre le Blanc, au spectrophotomètre à 450 nm / 630 ou 620 nm.

\*.i. t.a. température ambiante jusqu'au maximum à 25°C

## E DESARROLLO TEST ELISA

### Preparación de reactivos

#### Dilución de muestras:

Diluir 1:101 la muestra del paciente  
10 µl muestra + 1 ml [SPE]DIL.

En caso de pretratamiento de las muestras con absorbente RF, se debe tener en cuenta una dilución final de 1:101. Por favor, seguir las instrucciones para el uso del absorbente RF usado en el test.

Contrôles/calibradores son [RTU]!

Sacar los [MTS] necesarios de su envuelta y ponerlos en su soporte. No tocar los pocillos. Reponer los [MTS] desusados en la bolsa de plástico con desecante y cerrarla cuidadosamente

PIPETEAR 100µL DE BLANCO (A1), CONTROLES Y MUESTRAS DILUIDAS

PRIMERA INCUBACIÓN 30 MIN. A .i. t.a.\*

PRIMER LAVADO

Después de la incubación, lavar los pocillos 4 veces con [WASH]BUF

Pipetear 100 µl [CONJ]POD en cada pocillo

SEGUNDA INCUBACIÓN 30 MIN. A .i. t.a.\*

SEGUNDO LAVADO

Después de la incubación, lavar los pocillos 4 veces con [WASH]BUF

Pipetear 100 µl [SUBS]TMB en cada pocillo

TERCERA INCUBACIÓN 10 MIN. A .i. t.a.\*

Interrumpir la reacción exactamente después de 10 min., añadiendo 100µl de [SOLN]STOP en cada pocillo.  
Agitar la placa cuidadosamente hasta que el color sea homogéneo.

LEER EN FOTÓMETRO

Médir los valores de la densidad óptica (OD) después de 10 min. contra el blanco con un espectrofotómetro a 450 nm/630 o 620 nm.

\*.i. t.a. temperatura ambiente hasta máximo 25°C

## I TEST ELISA - PROCEDURA

### Preparazione dei reagenti

#### Diluzione dei campioni:

Diluire i campioni dei pazienti 1:101  
10 µl campione + 1 ml [SPE]DIL.

In caso di pretrattamento dei campioni con l'assorbente RF, deve essere considerata la soluzione finale di 1:101. Prego seguire le istruzioni per l'uso nell'assorbente RF usato nel test.

Controlli e calibratori sono [RTU]!

Prelevare dalla busta [MTS] necessarie e posizionarle sulla piastra. Non toccare i pozzetti. Le [MTS] non utilizzate vanno reinserite nella busta con l'essiccante e chiuse con cura.

PIPETTARE 100µL DI BIANCO (A1), CONTROLLI E CAMPIONI DILUITI

PRIMA INCUBAZIONE 30 MIN. A .i. t.a.\*

PRIMA FASE DI LAVAGGIO

Dopo l'incubazione lavare i pozzetti 4 volte con il [WASH]BUF

Pipettare 100 µl di [CONJ]POD in ogni pozzetto

SECONDA INCUBAZIONE 30MIN. A .i. t.a.\*

SECONDA FASE DI LAVAGGIO

Dopo l'incubazione lavare i pozzetti 4 volte con il [WASH]BUF

Pipettare 100 µl [SUBS]TMB in ogni pozzetto

TERZA INCUBAZIONE 10 MIN. A .i. t.a.\*

Dopo 10 min. esatti fermare la reazione con l'aggiunta di 100µl di [SOLN]STOP in ogni pozzetto. Agitare la piastra attentamente fino a che la colorazione diventa omogenea.

LETTURA FOTOMETRICA

Misurare tutti i valori di densità ottica (OD) entro 10 min. contro il bianco, con uno spettrofotometro a 450 nm/630 o 620 nm.

\*.i. t.a. temperatura ambiente fino al massimo a 25°C

## P TECNICA ELISA

### Preparação dos reagentes

#### Diluição das amostras:

Diluir as amostra 1:101  
10 µl amostra + 1 ml [SPE]DIL.

No caso de pré-tratamento das amostras com sorbente RF, deve considerar-se uma diluição final de 1:101. Por favor seguir as instruções de utilização do adsorvente RF utilizado no teste.

Controlos/Calibradores são [RTU]!

Retirar os necessários [MTS] da embalagem a colocar no suporte. Não tocar nos poços. Os [MTS] não utilizados devem ser colocados na embalagem com dessecantes e fechados cuidadosamente.

PIPETAR 100µL DE BRANCO (A1), CONTROLOS E AMOSTRAS DILUIDAS

PRIMEIRA INCUBAÇÃO 30 MIN. A .i. t.a.\*

PRIMEIRO PASSO DE LAVAGEM

Após incubação lavar 4 vezes com [WASH]BUF

Pipetar 100 µl [CONJ]POD em cada poço

SEGUNDA INCUBAÇÃO 30 MIN. A .i. t.a.\*

SEGUNDO PASSO DE LAVAGEM

Após incubação lavar 4 vezes com [WASH]BUF

Pipetar 100 µl [SUBS]TMB em cada poço

TERCEIRA INCUBAÇÃO 10 MIN. A .i. t.a.\*

Após exactamente 10 min. parar a reacção. Adicionar 100µl [SOLN]STOP em cada poço. Agitar a placa cuidadosamente até a cor homogénea.

LEITURA FOTOMÉTRICA

Medir todas as Densidades Ópticas (DO) no espaço de 10 min. contra o branco, com um espectrofotometro a 450 nm/630 ou 620 nm.

\*.i. t.a. temperatura ambiente até máxima 25°C

Titer der positiven Kontrolle oder Kalibratoren siehe Etiketten / titres of positive control or calibrators see labels

ELISA-Test  
(Semi-)Quantitatives Auswertungsdiagramm \* (semi-)quantitative evaluation diagram

