ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

CE GB

ELISA
ANTI - PARAINFLUENZA 1 IgG/ IgA

1. INTENDED USE

Enzyme-immunoassay for determination of antibodies against Parainfluenza Type 1 in human serum and plasma

IgG [REF] VEG 119 S 96 [IVD]
IgA [REF] VEA 119 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 patientõs sample are bound during the first incubation. After removing unbound material by washing, the presence of specific antibodies is detected using Anti-human IgG/ 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	IgA	Interpretation	Recommendation
-	-	no specific antibodies detectable	By suspect of acute infection ï control tests are recommendable
+	-	previous infection or reinfection	**Monitoring** of IgG antibodies (Sample collection within 10-14 days), a significant titre increase indicates an acute infection
++	-	recent previous infection, acute infection prob., reinfection poss.	**Monitoring** of IgG and IgA antibodies confirmatory tests e.g. IFA
-	+	primary infection probable	**Monitoring** of IgG and IgA antibodies(Sample collection within 10-14 days) to determine seroconversion; confirmatory tests e.g. IFA
++ +	+ +	acute infection, reinfection prob.	**Monitoring of** IgG and IgA antibodies confirmatory tests e.g. IFA

- negative, + positive, ++ positive (sample ≥ [CONTROL|+])

4. PERFORMANCE CHARACTERISTICS

Specificity / Sensitivity

80 samples IgG/ IgA were tested parallel in ELISA ANTI- PARAINFLUENZA 1 IgG/ IgA and comparison methods (ELISA). The sensitivity and specificity are based on the results found.

Specificity: IgG - **Sensitivity:** IgG 98,8%
Specificity: IgA 94,1% **Sensitivity:** IgA -

(In IgG test no sufficient negative samples were available)
(In IgA test no sufficient positive samples were available)

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 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 IgA samples was < 10%.

Cross-reactivity

Cross-reactivity with antibodies to Parainfluenza viruses Types 1-3 cannot be excluded. Close relationship exists between parainfluenza Type 1 and Type 3, between Parainfluenza Type 1, and Mumps and between Parainfluenza Type 2 and Types 1 and 3.

5. [Bib]

- Döller, G. in T. Porstmann, Diagn. Bibliothek, Vol.23 , Blackwell Verlag
- Selb, B.: Medizinische Virusdiagnostik, Umschau Verlag, Frankfurt

6. KIT COMPONENTS

1. 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.

2. PEROXIDASE CONJUGATE [CONJ|POD]
One vial containing 12 ml of ready-to-use anti-human IgG or IgA Peroxidase conjugate. Peroxidase conjugate contains 0.049% Thimerosal as preservative.

3. NEGATIVE CONTROL [CONTROL|-]
One vial of 1.2 ml containing human serum with 0,095% sodium azide as preservative. Ready to use.

4. CUT OFF CONTROL [CUTOFF]
One vial of 1.2 ml containing human serum with 0,095% sodium azide as preservative. Ready to use.

5. 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 ≥ 1,500.

6. TMB SUBSTRATE [SUBS|TMB]
One vial containing 13 ml of ready-to-use tetra-methylbenzidine (TMB) substrate.

7. 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.

8. WASH SOLUTION 25X [WASH|BUF|25x]
One bottle containing 80 ml (2x40 ml) of wash solution concentrate.

9. STOP SOLUTION [SOLN|STOP]
One bottle containing 15 ml of 0.95N H_2SO_4 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.

1. Only qualified and well-trained employees should carry out the assay procedure.
2. 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.
3. Do not mix lot specific reagents, such as [MTS], controls and [CONJ|POD] from different kit lots. The [SUBS|TMB] doesn't 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.
4. 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.
5. The [MTS] are coated with inactive antigen. However, normal laboratory precautions should be maintained when handling with infectious material. Do not pipette by mouth.
6. 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.
7. Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.
8. The [SOLN|STOP] (0,95 N H_2SO_4) contains sodium hydroxide which may irritate skin and mucous membranes.Wash thoroughly with water in case of contact.
9. For disposal the legal regulations have to be followed.

10. SPECIMEN COLLECTION AND STORAGE

1. Microbially contaminated specimen may cause interference.
2. Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
3. Suitable specimens are serum or plasma (heparinized, EDTA) samples obtained by standard laboratory techniques.
4. The samples should not be heat-inactivated since non-specific results may occur.
5. Results on tests using CSF are not available.
6. 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.
7. **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.

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

A. Pipette 100µl of the controls or diluted patient sample into the wells. Pipette 100 µl of sample diluent into well A1 (Blank).

B. Incubate the wells at room temperature (21-25°C) for 30 minutes, protected from intense light.

C. Wash the wells four times as described in section k. WASHING PROCEDURE

D. Add 100µl of ready-to-use peroxidase conjugate to each well.

E. Incubate the wells at room temperature (21-25°C) for 30 minutes, protected from intense light.

F. Repeat washing as in section C above.

G. Add 100µl of ready-to-use TMB substrate to each well.

A. Incubate the wells at room temperature (21-25°C), in the dark for 10 minutes

B. Add 100µl of stop solution to each well. Tap gently to ensure homogenous color distribution and read within 10 minutes.

C. 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 substracted 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 extend.

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 ≥ 1.5
- OD-value of the Blank should not be higher than 0,100.

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 ñCut-off Valueò

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.

Cut-off Value = OD of the Negative Control + OD of the Cut-off Control

CUT-OFF RANGE = CUT-OFF VALUE +/- 10%

Interpretation of sample results:

RESULT	DEFINITION
negative -	OD value sample < Cut-off value **-10%**
equivocal	OD value sample ≥ Cut-off value **-10%** OD value sample ≤ Cut-off value **+10%**
positive +	OD value sample > Cut-off value +**10%**

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

B CALCULATION OF RATIO (CUT-OFF INDEX, COI):

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

COI = OD value of sample/ 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-PARAINFLUENZA 1 IgA** is a *positive reference sample* with a **ratio ≥ 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.**

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

Símbolos/ Simboli/ Simbolos/ Symboly/ Címkékén/ Ɇgɛƃɑǝ̈Ŭ IVD

[MTS]	Mikrotiterstreifen/ microtiterstrips/ Microplaques sensibilisèes/ placa de microtítulo/ piasta microtitolo/ Tiras de microtitulação/ Mikrotitraḷ ní stripy/ Mikrotitercsíkok/ ȼŬɑɑŮɠ ɛɑɟ ɑŬ́ŭɑ ́ ɑɑŮɗɠ
[SPE|DIL]	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ soluzione tampone/ estabilizador de diluição/ ř edicí na vzorku/ Mintahígító/ ɟ ɟ Ŭɑɤ Ŭɑɦ ɑŮɒɛŬŬɑɠ
[WASHBUF|25x]	Waschlösung/ wash solution/ solution de lavage/ solución limpiadora/ soluzione lavaggio/ solução de lavagem Konzentrat/ concentrate / Concentré / concentrado/ concentrato/ concentrado 25x/ Promývací roztok 25x/ Mosópufferkoncentrátum 25x/ ǣɑɤǝɡµŬ Ʉɑɨ ůɗɠ 25x
[CONJ|POD]	Peroxidase-Konjugat / Peroxidase conjugate / Conjugue Peroxidase/Conjugado Peroxidasa / coniugato con perossidasi/ conjugado Peroxidase/ Peroxidázový konjugát / Peroxidáz Konjugátum/ Ɇgɑ Ůɡɔɛɏɜɗ ɡ ́ Ů ɑɜŮɑɤůɗ
[CONTROL|-]	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo / Negativní kontrola/ Negatív Kontroll/ Ŭɟ ɜɗŬɑɦɠ ɀ ɤɟ Ŭɡɟ Ŭɠ
[CUT_OFF]	Cut-off Kontrolle/ cut-off control/ Contrôle cut-off/ control valor límite/ controllo limitante/ controle interrupção/ Cut off kontrola/ cut off kontroll/ ɛɤɟ Ŭɡɟ ŬɠŬ́ ɑǝɑ ́ ɐɠ
[CONTROL|+]	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo/ Pozitivní kontrola/ pozitív kontroll/ ɗŮŬɑɦɠ ɛɤɟ Ŭɡɟ Ŭɠ
[SUBS|TMB]	TMB-Substrat/ TMB substrate/ substrat TMB/ substrato TMB / TMB Substrát / TMB Szubsztrát/ Ɉ ́ ɦůŲ ɤ µŬ TMB
[SOLN|STOP]	Stopplösung/ stop solution/ Solution d'arrêt/ solución de parada/ soluzione d'arresto/ solução de parada/ Stop pufr/ Stop Oldat/ ǣɑɤǝɡµŬ ȼ Ů µŬɑɑɑ µɑɨ
[Bib]	Literatur/ Literature/ Littérature/ Bibliografia/ Bibliografía/ Literatura/ Irodalom/ ɑɑɑǝɑɔɟ Ŭɑɑ Ŭ
[LOT]	Charge/ lot/ Lot/ lote/ carcia/ lote/ Ḷ íslo ġarġe/ Lot Szám/ ɟ ɟ ɑɑµɦɠ ́ Ŭɟ ŬɑɑŬɠ
[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/ ǣɑŬɔɜɤ ůŬɑɐ ɑŬŲ ɑɐ ůɡůǝŮɡɐ 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 SzereplṘ Kód/ ȽɤŭɑɦɠȽŬŬŬɑɦɔɑɡ
S 96	96 Bestimmungen/ tests/ testŜ/ determinazioni/ testes / Poḷ et testŢ/ Vizsgálatok Száma/ ɟ ɟ ɑɑµɦɠ ŮɑŮŬɤŮŮɤ ɜ
I	Gebrauchsanweisung beachten/ consult instructions for use/ consulter le mode d'emploi/consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruçõesde uso/ PŠeḷ tŊte si Návod k pouġití/ Olvassa el a Használati Utasítást/ ǣŮŬŬŬɃ ŭɗɔɑŮɠ ɟ ɟ ɐůŮɤ ɠ
t	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura/ Teplotní limuty/ HṘmérsékleti Korlátozások/ ŬŮ µɑǝɟ ŬůɑŬǝɤ ɦɟ ɑŬ
e	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade/ Datum exspirace/ Lejárati IdṘ/ ȼ µŮ ɑµɗɜɑŬ ǝɜɗɠ (ɟ ɟ ɐůɗ ɏɤ ɠ ...)
M	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por/ Výrobce/ Gyártó/ ȽŬŬŬůǝŮɡɤɑŮŬŬǝŬ ́ ɦ...

9/2008

M :

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Symbole nach IVD/ symbols used with IVD devices/

Symbole / Símbolos / Simboli / Simbolos IVD

MTS	Mikrotiterstreifen/ microtiterstrips/ Microplaques sensibilisées/ placa de microtítulo/ piasta microtitolo/ placa do microtitre
SPE DIL	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ soluzione tampone/ estabilizador de diluição
WASHBUF 25x	Waschlösung/ wash solution/ solution de lavage/ solución limpiadora/ soluzione lavaggio/ solução de lavagem Konzentrat/ concentrate / Concentré / concentrado/ 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	Stoplösung/ stop solution/ Solution d'arrêt/ solución de parada/ soluzione 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 límite/ controllo limitante/ controle interrupção
CAL 1	Calibrator/ calibrator/ calibrateur/ calibrador/ calibratori/ calibrador 1
CAL 2	Calibrator/ calibrator/ calibrateur/ calibrador/ calibratori/ calibrador 2
CAL 3	Calibrator/ calibrator/ calibrateur/ calibrador/ calibratori/ calibrador 3
CAL 4	Calibrator/ calibrator/ calibrateur/ calibrador/ calibratori/ 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/ liso para su uso/ pronto per l'uso/ pronto para usar
96	96 Bestimmungen/ tests/ testś/ determinazioni/ testes
	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
	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura
	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade
	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por

9/2008

(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. R.T.*

▼

1. WASCHSCHRITT

▼

Nach 1. Inkubation **4 mal** mit hergestellter WASHBUF waschen

▼

100 µl CONJ POD je Vertiefung pipettieren

▼

2. INKUBATION 30 MIN. R.T.*

▼

2.WASCHSCHRITT

▼

Nach 2. Inkubation **4 mal** mit hergestellter WASHBUF waschen

▼

100 µl SUBS TMB je Vertiefung pipettieren

▼

3. INKUBATION 10 MIN. 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.**
* 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 *

▼

FIRST WASH STEP

▼

After incubation wash the wells **4 times** with WASHBUF

▼

Pipette **100 µl** CONJ POD each well

▼

**SECOND INCUBATION 30 MIN. room *

▼

SECOND WASH STEP

▼

After incubation wash the wells **4 times** with WASHBUF

▼

Pipette **100 µl** SUBS TMB each well

▼

**THIRD INCUBATION 10 MIN. room *

▼

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 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 du pretraitement 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 t.a.*

▼

PREMIER LAVAGE

▼

Laver **4 fois** les puits avec
[WASHBUF]

▼

Déposer **100 µl** de [CONJ POD] dans chaque puits

▼

DEUXIÈME INCUBATION 30 MIN. À t.a.*

▼

DEUXIÈME LAVAGE

▼

Laver **4 fois** les puits avec
[WASHBUF]

▼

Déposer **100 µl** de [SUBS TMB] dans chaque puits

▼

TROISIÈME INCUBATION 10 MIN. À 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.

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

9/2008

(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 instrucciónes para el uso del absorbente RF usado en el test.
Contróles/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 t.a.*

▼

PRIMER LAVADO

▼

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

▼

Pipetear **100 µl** [CONJ POD] en cada pocillo

▼

SEGUNDA INCUBACIÓN 30 MIN. A t.a.*

▼

SEGUNDO LAVADO

▼

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

▼

Pipetear **100 µl** [SUBS TMB] en cada pocillo

▼

TERCERA INCUBACIÓN 10 MIN. A t.a.*

▼

Interrumpir la reacción **exactamente** despues 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) despues de **10 min.** contra el blanco con un espectrofotómetro a 450 nm/630 o 620 nm.

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

(I) TEST ELISA - PROCEDURA

Preparazione dei reagenti

Diluizione 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 t.a.*

▼

PRIMA FASE DI LAVAGGIO

▼

Dopo l´incubazione lavare i pozetti 4 volte con il [WASHBUF]

▼

Pipettare **100 µl** di [CONJ POD] in ogni pozzetto

▼

SECONDA INCUBAZIONE 30MIN.A t.a.*

▼

SECONDA FASE DI LAVAGGIO

▼

Dopo l´incubazione lavare i pozetti 4 volte con il [WASHBUF]

▼

Pipettare **100 µl** [SUBS TMB] in ogni pozzetto

▼

TERZA INCUBAZIONE 10 MIN. A t.a.*

▼

Dopo **10 min. essati** 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.

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

(P) TÉCNICA ELISA

Preparação dos reagentes

Diluição das amostras:
Diluir as amostra **1:101**
10 µl amostra + 1 ml [SPE DIL].
No caso de prétramento das amostras com absorvente 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 neccesários [MTS] da embalagem a colocar no suporte. Nào 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 t.a.*

▼

PRIMEIRO PASSO DE LAVAGEM

▼

Após incubação lavar 4 vezes com [WASHBUF]

▼

Pipetar **100 µl** [CONJ POD] em cada poço

▼

SEGUNDA INCUBAÇÃO 30 MIN. A t.a.*

▼

SEGUNDO PASSO DE LAVAGEM

▼

Após incubação lavar 4 vezes com [WASHBUF]

▼

Pipetar **100 µl** [SUBS TMB] em cada poço

▼

TERCEIRA INCUBAÇÃO 10 MIN. A t.a.*

▼

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

▼

LEITURA FOTOMÉTRICA

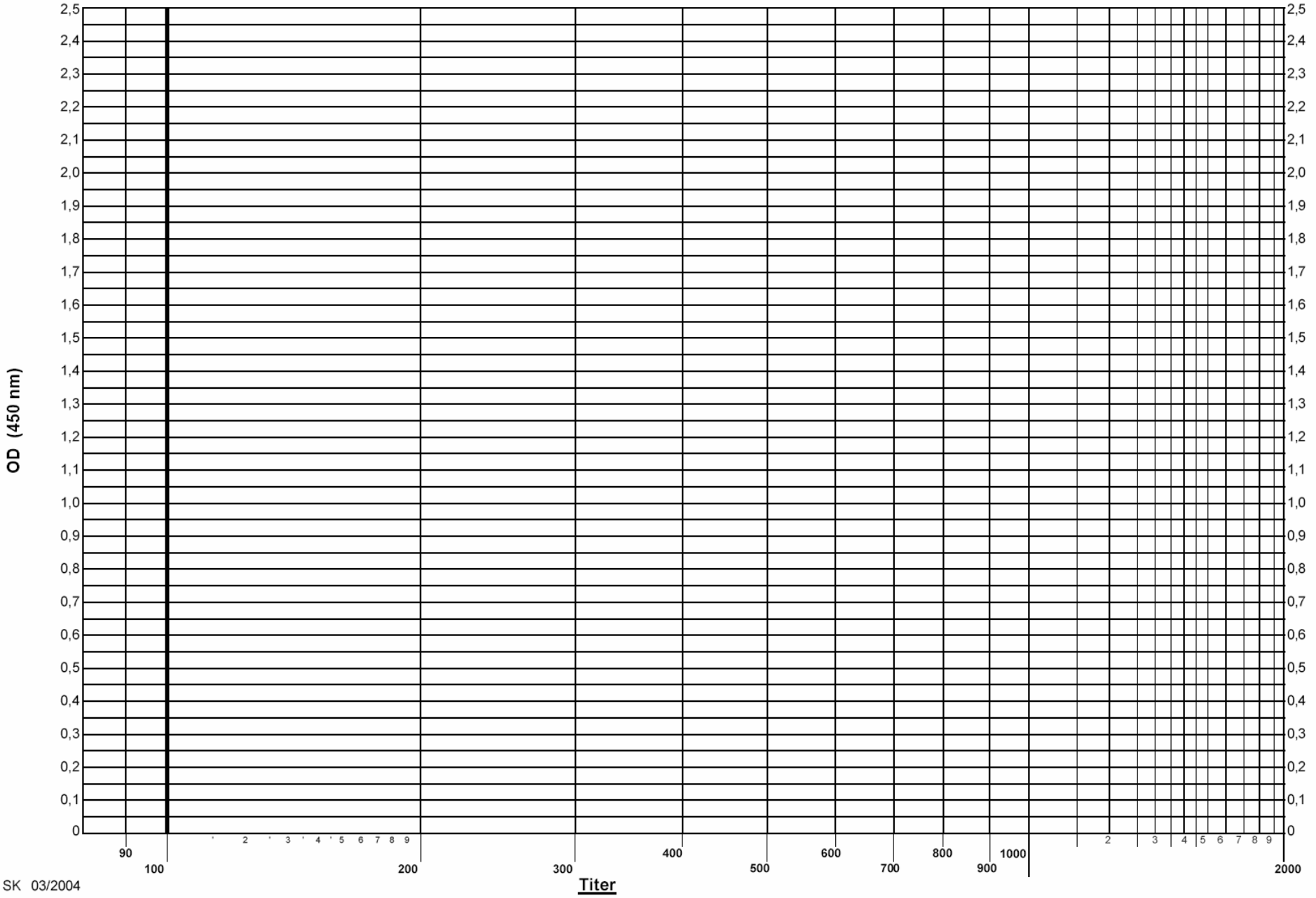
▼

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

*t.a. temperatura ambiente até màxima 25°C

ELISA-Test

(Semi-)Quantitatives Auswertungsdiagramm * (semi-)quantitative evaluation diagram



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

SK 03/2004