AESKULISA 25OH Vitamin D Total

Ref 3810

Instruction Manual

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1 Intended Use

AESKULISA 25OH Vitamin D Total is a solid phase enzyme immunoassay for the in vitro quantitative measurement of 25-hydroxyvitamin D_2 and D_3 (25(OH) D_2 and 25(OH) D_3) in serum. The assay is a tool for the determination of serum Vitamin D status.

2 Clinical Application and Principle of the Assay

Vitamin D is an essential steroid hormone well known for its role in calcium homeostasis and bone metabolism. Moreover, it is involved in a number of physiological processes. Insufficient levels of Vitamin D are associated with skeletal pathologies like rickets, osteoporosis and osteomalacia. Recent studies indicate an correlation of Vitamin D deficiency and a number of non-skeletal disorders including cardiovascular, autoimmune and infectious diseases, diabetes and cancer. Vitamin D intoxication occurs very rarely but can lead to vascular and tissue calcification, with subsequent damage to the heart, blood vessels, and kidneys. In pregnancy, a Vitamin D deficiency may affect the predisposition of the fetus to develop chronic diseases.

There are two isomeric forms of Vitamin D, Vitamin D_2 (Ergocalciferol) and Vitamin D_3 (Cholecalciferol). Whereas food products like fatty fish and milk products contain both forms, Vitamin D_3 is additionally produced in the skin from sun exposure. In the liver, it is converted into 25-hydroxyvitamin D (25(OH)D), the major circulating form. Both Vitamin D and 25(OH)D are bound to the Vitamin D binding protein (VDBP) in the circulation. However, 25(OH)D is biologically inactive and has to be metabolized to its biologically active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in the kidneys by a tightly regulated mechanism.

The serum level of 25-hydroxyvitamin D (representing D_2 and/or D_3) has been widely accepted as useful biomarker for the determination of the Vitamin D status (deficiency, insufficiency, intoxication) and as a therapy control. Based on studies worldwide, it has been estimated that 1 billion people have Vitamin D levels below the normal range. Especially, people with limited sun exposure (chronically ill or care dependent individuals, ethnic and religious groups with whole body clothing), non-Caucasian and babies represent risk groups for Vitamin D deficiency.

For the determination of 25-hydroxyvitamin D amounts, methods like liquid chromatography , mass spectrometry, radioimmunoassays , enzyme immunoassays , competitive protein binding assays and chemiluminescent immunoassays are routinely used.

Principle of the test

The **AESKULISA 25OH Vitamin D Total** is a solid phase Enzyme Linked Immuno-sorbent Assay performed on microtiterplates. Serum samples are incubated in the wells allowing the total 25OH Vitamin D (D_2 and D_3) present in the serum to dissociate from binding serum proteins and bind to a monoclonal antibody. After a washing step, a defined amount of biotin-labeled 25OH Vitamin D in presence of horseradish peroxidase (HRP) competes with unlabeled serum 25OH Vitamin D bound to the monoclonal antibody. The excess of biotin-labeled 25OH Vitamin D is washed off after the incubation. Addition of TMB-substrate generates an enzymatic colorimetric reaction (blue), which is stopped by addition of diluted acid (color changes to yellow). The intensity of the color formation after the chromogenic reaction is a function of the amount of biotin-labeled 25OH Vitamin D bound to the monoclonal antibody and is inversely proportional to the concentration of 25OH Vitamin D_3 and D_2 present in the samples. The concentration of the 25OH Vitamin D_3 and D_2 present in the samples is extrapolated by dose interpolation using the calibration curve obtained from the absorbance of the standards.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Calibrator A	1 x lyophilized	White	N/A	Biological matrix with gentamycin and proclin
Calibrator B-F	5 x lyophilized	White	N/A	Calibrators B-F in horse serum with gentamycin and proclin
Control D (Con D)	1 x lyophilized	Red	N/A	human deficient serum
Control N (Con N)	1 x lyophilized	Green	N/A	human normal serum
Concentrated Conjugate	1 x 0.2 ml	Blue	Orange	100x concentrated 25OH Vitamin D conjugated with biotin
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Incubation Buffer	1 x 20ml	Green	Colorless	casein
Conjugate	1 x 20ml	Blue	Colorless	casein and streptavidin HRP
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	Refer to paragraph 2
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and reference filter (600-690nm). Glassware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, plate shaker (400-700rpm), precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted calibrators and controls are stable at 2-8°C/35-46°F for 8 weeks. For longer storage periods, aliquots should be stored at -20°C for no longer than 3 months. Avoid subsequent freeze-thaw cycles. Reagents and the microplate should be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in original bag, including the desiccant, and seal tightly. The working wash solution and the conjugate solution have to be prepared fresh every time.

5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect, please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at 23°C/73.4°F for automated systems.

Never expose components to higher temperature than 37°C/ 98.6°F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

It is recommended to run calibrators, controls and samples in duplicate.

To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to 10 minutes maximum.

Prepare a calibration curve for each run. Do not use data from previous runs.

Dispense the chromogenic solution within 15 minutes following the washing of the microtiterplate.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8h. Alternatively, serum samples should be stored tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods.

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated reagents:

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Prepare calibrators and controls:

Calibrator A-F: reconstitute the calibrators with 1 ml distilled water each.

Controls Con D and Con N: reconstitute the controls with 1 ml distilled water each.

Mix thoroughly for complete solubilisation by using a vortex or rotation mixer.

Prepare the working conjugate solution:

The working conjugate solution has to be prepared before the sample incubation step has started.

Prepare only as much working conjugate solution as needed for each run by dilution of concentrated conjugate (100x) with conjugate in a 1:100 ratio, according to the number of strips (e.g. manual processing, 6 strips: dilution of 50 µl concentrated conjugate with 5 ml conjugate).

Please consider respective additional volume when using automated systems.

Use a vortex to homogenize. Keep the working HRP conjugate solution at room temperature and avoid direct exposure to sunlight. Diluted conjugate has a limited shelf life! Do not use residual solutions at a later date.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 350 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice.

Microtiterplates:

Calculate the number of strips required for the test. Remove unused strips from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

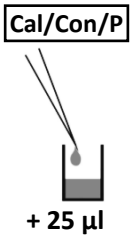
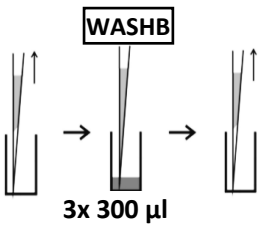
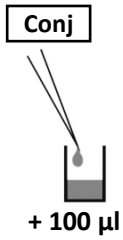
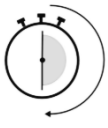
7.2 Pipetting Scheme

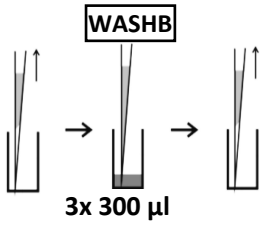
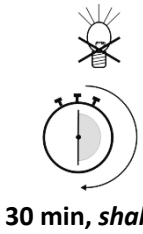
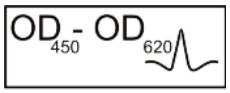
We recommend to pipette calibrators, controls and samples as follows:

	1	2	3	4...
A	CalA	CalE	P1	
B	CalA	CalE	P1	
C	CalB	CalF	P2	
D	CalB	CalF	P2	
E	CalC	ConD	P3	
F	CalC	ConD	P3	
G	CalD	ConN	…	
H	CalD	ConN	…	

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C
CalD: calibrator D
CalE: calibrator E
CalF: calibrator F
Con D: Control D
Con N: Control N
P1: patient 1
P2: patient 2
P3: patient 3

7.3 Test Steps

Step	*Description*	
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting. Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme.	
	CALIBRATORS, CONTROLS & SAMPLES	
2.		Pipette into the designated wells, as described in chapter 7.2 above, 25 µl of either: • Calibrators (CalA to CalF) • Control D (ConD), Control N (ConN), and • Undiluted Patients serum (P1, P2...)
3.		Pipette 75 µl of Incubation Buffer into **all** wells.
4.		Incubate for 1 hour at room temperature **on a plate shaker (400 to 700 rpm)**.
5.		Aspirate the liquid from each well and wash the plate three times with 300 µl of washing buffer (diluted 1:50). Aspirate the liquid after each washing cycle.
	CONJUGATE	
6.		Pipette 100 µl of working conjugate solution into each well.
7.		Incubate for 30 minutes at room temperature **on a plate shaker (400 to 700 rpm)**.

8.		Aspirate the liquid from each well and wash the plate three times with 300 µl of washing buffer (diluted 1:50). Aspirate the liquid after each washing cycle.
	SUBSTRATE	
9.		Pipette 100 µl of TMB substrate into each well within 15 minutes after the washing step.
10.		Incubate for 30 minutes at room temperature **on a plate shaker (400 to 700 rpm)** protected from intense sunlight.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Agitate plate carefully for 5 sec, incubate for 5 minutes at RT
13.		Read absorbance at 450 nm (recommended reference filter 620 nm) within 30 minutes and calculate the results as described in section 8.

8 Calculation of results

Plotting the calibration curve

To plot the calibration curve, calculate the mean optical density of each calibrator, control and sample. For each calibrator, control and sample, calculate the following (B0 = absorbance Calibrator A, B = absorbance):

$$B/B0(\%) = \frac{OD\ (Calibrator, Control, Sample)}{OD\ (Calibrator\ A)} \times 100$$

To establish a standard curve, the values B/B0 (%) of each calibrator are plotted against the corresponding 25OH Vitamin D concentration using either linear-linear of semi-logarithmic graph paper. By interpolation of the sample B/B0 (%) values, determine the 25OH Vitamin D concentration of the samples using the calibration curve.

Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

Example of a standard curve

For every determination, please perform a new calibration curve. Please note that the concentrations of the calibrators are lot specific.

Do not use this example to evaluate patient sera!

Calibrator	Concentration	OD units
A	0 ng/ml	2.25
B	6 ng/ml	2.07
C	13 ng/ml	1.83
D	23 ng/ml	1.43
E	52 ng/ml	0.78
F	130 ng/ml	0.25

1 ng/ml = 2.496 pmol/ml[a]

Evaluation of the values

25OH Vitamin D levels are normally affected by dietary intake, season, climate zone, age, skin colour and genetic background. Each laboratory should establish its own range based on their local population.

The German Bundesinstitut für Risikobewertung (BfR) recommend in accordance with the Deutsche Gesellschaft für Ernährung (DGE), the World Health Organization (WHO), the Institute of Medicine (IOM) and the National Institute of Health (NIH) the following reference ranges for the serum concentration of 25OH Vitamin D [b, c].

severe deficiency	< 12 ng/ml	< 30 nmol/l
insufficiency	12 - 20 ng/ml	30 - 50 nmol/l
sufficiency	≥ 20 ng/ml	≥ 50 nmol/l
Vitamin D intoxination	> 160 ng/ml	> 400 nmol/l

[a] SI conversion factor: to convert 25(OH)D values to nmol/l, multiply by 2.496.

[b] German Nutrition Society: Ann. Nutr. Metab., 60:241–246, 2012

[c] Institute of Medicine, Food and Nutrition Board: Dietary Reference Intakes for Calcium and Vitamin D.

Washington, DC: National Academy Press, 2010.

9 Technical Data

Sample material:	serum
Sample volume:	25 µl, undiluted
Total incubation time:	2 hours at room temperature under shaking
Calibration range:	0 - 130 ng/ml (0 - 325 nmol/l), lot specific
Analytical sensitivity:	3.8 ng/ml
Storage:	2-8°C/35-46°F in original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Analytical sensitivity

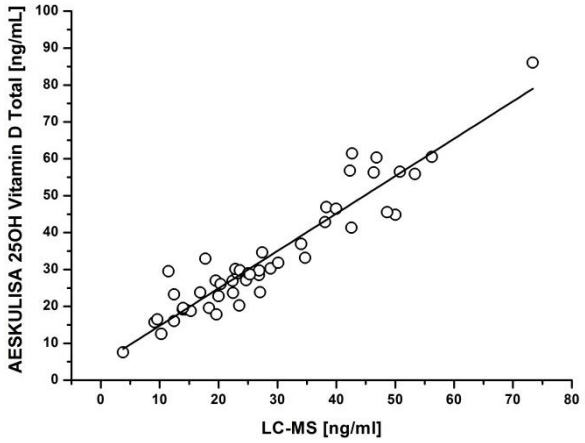
The analytical sensitivity has been determined according to the CLSI-guideline EP17-A at 3.8 ng/ml.

10.2 Performance data

The AESKULISA Vitamin D total ELISA test was compared to the LC-MS method (liquid chromatography–mass spectrometry) and a Chemiluminescent Microparticle Immunoassay (CMIA, ARCHITECT 25-OH Vitamin D, Abbott). The correlation of these tests to the AESKULISA 25OH Vitamin D Total was determined by a linear regression of the data.

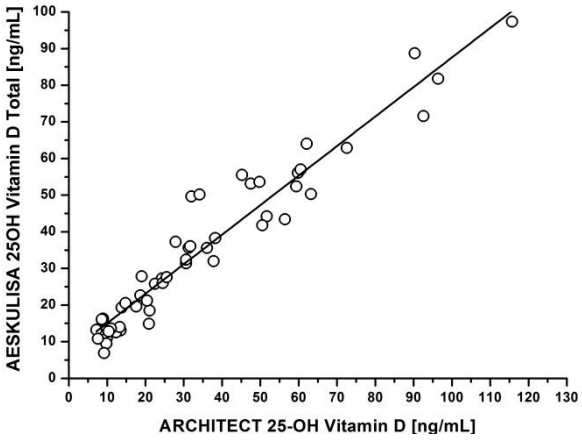
Method comparison versus LC-MS

Samples (n)	Slope	Intercept	R^2
49	1.01	4.6	0.888



Method comparison versus ARCHITECT 25-OH Vitamin D, Abbott

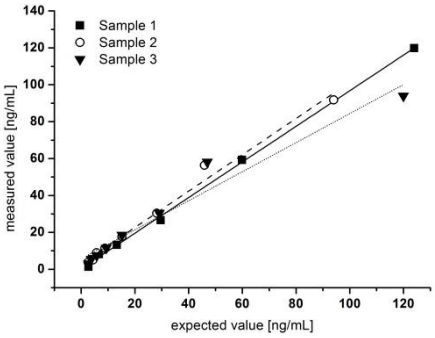
Samples (n)	Slope	Intercept	R^2
51	0.806	6.9	0.927



10.3 Linearity

To determine the linearity of the AESKULISA 25OH Vitamin D Total, serial dilutions of sera were measured. The obtained results were compared to the expected ones, which were calculated by the quotient of measured value of the next higher concentration and the dilution

factor 2. Recovery is the percentage of the measured value to the expected one. Additionally, a linear regression was performed.

No.	Dilution Factor	Measured (ng/ml)	Expected (ng/ml)	Recovery (%)	Linear Regression y = b x + a		Graph
1	1	119.8	124.0	97%	b	0.96	
	1 / 2	59.2	59.9	99%	a	0.27	
	1 / 4	26.6	29.6	90%	R^2	0.99	
	1 / 8	13.2	13.3	99%			
2	1	119.6	125.0	96%	b	0.97	
	1 / 2	71.9	59.8	120%	a	2.98	
	1 / 4	36.6	35.9	102%	R^2	0.98	
	1 / 8	19.2	18.3	105%			
3	1	91.8	94.0	98%	b	0.98	
	1 / 2	56.3	45.9	123%	a	2.99	
	1 / 4	30.4	28.2	108%	R^2	0.98	
	1 / 8	17.7	15.2	117%			

10.4 Precision

To determine the precision of the assay, the variability (intra- and inter-assay) was assessed by examining its reproducibility on serum samples selected to represent the standard curve.

Intra-assay		
Sample No.	Mean (ng/ml)	CV (%)
1	18.1	7
2	38.2	5
3	50.7	5
4	127.1	3

Inter-assay		
Sample No.	Mean (ng/ml)	CV (%)
1	18.2	13
2	38.2	6
3	50.7	6
4	127.1	7

10.5 Recovery

Recovery was determined spiking known amount of 25OH Vitamin D_3 and 25OH D_2 into human serum. Mean recoveries are reported in the table below.

	ng/ml	Recovery (%)
25OH-Vitamin D3	16.9	107
25OH-Vitamin D2	27.8	86

10.6 Influence of Interfering Substances

The microplate is coated with a monoclonal anti Vitamin D antibody. Cross-reactivity has been tested against the substances reported in the table below and the respective recovered values were calculated.

Substance	Recovery (%)
Vitamin D3 (Cholecalciferol)	108
Vitamin D2 (Ergocalciferol)	103
3-epi-25-Hydroxyvitamin D3	92
Hemoglobin (5 g/l)	97
Bilirubin (0.5 g/l)	103
Bilirubin conjugate (1 g/l)	102
Triglycerides (5 g/l)	101

11 Literature

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IVD	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
REF	¨ Numero d'ordine	¨ Cataloge number
	¨ Référence Catalogue	¨ Numéro de catálogo
	¨ Bestellnummer	¨ Αριθμός παραγγελίας
	¨ Número de catálogo	
LOT	¨ Descrizione lotto	¨ Lot
	¨ Lot	¨ Lote
	¨ Chargen Bezeichnung	¨ Χαρακτηρισμός παρτίδας
	¨ Lote	
CE	¨ Conformità europea	¨ EC Declaration of Conformity
	¨ Déclaration CE de Conformité	¨ Declaración CE de Conformidad
	¨ Europäische Konformität	¨ Ευρωπαϊκή συμφωνία
	¨ Déclaracão CE de Conformidade	
Σ 96	¨ 96 determinazioni	¨ 96 tests
	¨ 96 tests	¨ 96 pruebas
	¨ 96 Bestimmungen	¨ 96 προσδιορισμοί
	¨ 96 Testes	
i	¨ Rispettare le istruzioni per l'uso	¨ See instructions for use
	¨ Voir les instructions d'utilisation	¨ Ver las instrucciones de uso
	¨ Gebrauchsanweisung beachten	¨ Λάβετε υπόψη τις οδηγίες χρήσης
	¨ Ver as instrucões de uso	
	¨ Da utilizzarsi entro	¨ Use by
	¨ Utilise avant le	¨ Utilizar antes de
	¨ Verwendbar bis	¨ Χρήση μέχρι
	¨ Utilizar antes de	
+2°C +8°C	¨ Conservare a 2-8°C	¨ Store at 2-8°C (35-46°F)
	¨ Conserver à 2-8°C	¨ Conservar a 2-8°C
	¨ Lagerung bei 2-8°C	¨ Φυλάσσεται στους 2-8°C
	¨ Conservar entre 2-8°C	
	¨ Prodotto da	¨ Manufactured by
	¨ Fabriqué par	¨ Fabricado por
	¨ Hergestellt von	¨ Κατασκευάζεται από
	¨ Fabricado por	
INCB	¨ Buffer di incubazione	¨ Incubation buffer
	¨ Tampon d'incubation	¨ Tampón de incubación
	¨ Inkubations Puffer	¨ ρυθμιστικό διάλυμα επώασης
	¨ Tampão de incubação	
CON D	¨ Siero di controllo Deficiente	¨ Deficient control serum
	¨ Sérum de contrôle insuffisantes	¨ Suero de control deficiente
	¨ Defizientes Kontrollserum	¨ Ελλιπή ορό ελέγχου
	¨ Soro de controlo deficiente	
CON N	¨ Siero di controllo normale	¨ Normal control serum
	¨ Sérum de contrôle normal	¨ Suero de control normal
	¨ Normales Kontrollserum	¨ Φυσιολογικό ορό ελέγχου
	¨ Soro de controlo normal	
CAL	¨ Calibratore	¨ Calibrator
	¨ Etalon	¨ Calibrador
	¨ Kalibrator	¨ Αντιδραστήριο βαθμονόμησης
	¨ Calibrador	
CONJ	¨ Coniugato	¨ Conjugate
	¨ Conjugé	¨ Conjugado
	¨ Konjugat	¨ Σύζευγμα
	¨ Conjugado	
CONJ conc	¨ coniugato concentrato	¨ Concentrated conjugate
	¨ conjugué concentré	¨ conjugado concentrado
	¨ Konzentriertes Konjugat	¨ συγκέντρωσης σύζευξη
	¨ conjugado concentrado	
MP	¨ Micropiastra rivestita	¨ Coated microtiter plate
	¨ Microplaque sensibilisée	¨ Microplaca sensibilizada
	¨ Beschichtete Mikrotiterplatte	¨ Επικαλυμμένη μικροπλάκα
	¨ Microplaca revestida	
WASHB 50x	¨ Tampone di lavaggio	¨ Wash buffer
	¨ Tampon de Lavage	¨ Solución de lavado
	¨ Waschpuffer	¨ Ρυθμιστικό διάλυμα πλύσης
	¨ Solucão de lavagem	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ρυθμιστικό διάλυμα υποστρώματος
	¨ Substrato	
STOP	¨ Reagente bloccante	¨ Stop solution
	¨ Solution d'Arrêt	¨ Solución de parada
	¨ Stopreagenz	¨ Αντιδραστήριο διακοπής αντίδρασης
	¨ Solucão de paragem	
add	¨ aggiunta di	¨ addition of
	¨ addition d'	¨ Además de
	¨ Zugabe von	¨ προσθήκη
	¨ adição de	