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INSTRUCTION MANUAL

AESKULISA® 25OH Vitamin D

Ref 3810

CE | Σ 96 | 2°C – 8°C | IVD | eIFU WWW.AESKU.COM

Instruction Manual

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1 Intended Use

The ***AESKULISA®*** **25OH Vitamin D** ELISA is a solid phase enzyme immunoassay for the quantitative determination of 25-hydroxy vitamin D and other hydroxylated metabolites in human serum. The test is for identifying the overall status of vitamin D.

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, sufficiency and 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 calibrators, controls and samples are diluted with biotin-marked 25(OH)D and then incubated in the wells of the ***AESKULISA®*** **25OH Vitamin D**, which are coated with a highly specific 25(OH)D antibody. 25(OH)D from the patient's serum and the biotin-marked 25(OH)D compete with each other to bind with the antibody on the plate. Non-bound serum components are washed away in the following washing step. Then avidin marked with horseradish peroxidase that selectively binds to biotin complexes is added. Another washing step is followed by an enzymatic color reaction (blue) using the substrate (TMB), which is stopped using diluted acid (sudden color change to yellow). The intensity of the chromogen color is inversely proportional to the 25(OH)D concentration in the serum.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
25(OH)D biotin concentrate 50x	1 x 1ml	Yellow	N/A	50x concentrate Lyophilized buffer with biotin-marked 25(OH)D and proprietary stabilizers
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Control 1	1 x 1ml	Red	Light yellow	Human serum: contains 25(OH)D and 0.09% sodium azide (preservative)
Control 2	1 x 1ml	Green	Light yellow	Human serum: contains 25(OH)D and 0.09% sodium azide (preservative)
Calibrators A-G	7 x 1ml	White	Light yellow	Buffered human serum: contains 25(OH)D and 0.09% sodium azide (preservative)
25(OH)D buffer	1 x 50ml	Yellow	Yellow	Proprietary reagent for dissociating 25(OH)D from its ligands
Conjugate	1 x 15ml	Blue	Blue	Avidin-marked horseradish peroxidase, protein, enzyme stabilizers and preservatives
TMB substrate	1 x 15ml	Black	Colorless	Stabilized TMB/H2O2
Stop solution	1 x 15ml	White	Colorless	1M hydrochloric acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), Borosilicate glass or polypropylene test tubes for dilutions, test tubes for dilutions. Vortex mixer, 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/35.6–46.4 °F, in their original containers. Reconstituted 25(OH)D biotin solution has a shelf life of 4 weeks after use when protected from light at 2–8 °C/35.6–46.4 °F. After being opened, the calibrators and controls have a shelf life of 4 weeks at 2–8 °C/35.6–46.4 °F. Diluted washing solution can be kept for one month at 2–8 °C/35.6–46.4 °F. 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.

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 (18-25 °C/64.4-77 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at maximum 25 °C/77 °F for automated systems.

Never expose components to higher temperature than 30 °C/86 °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.

Do not use polystyrene test tubes for ***AESKULISA®* 25OH Vitamin D.**

After diluting the samples, calibrators, and controls with the biotin solution, they must be pipetted into the wells within 30 minutes.

To ensure that the temperature of all the plates remains the same, do not stack the plates during incubation.

Cover/seal the plates during incubation.

A definitive clinical diagnosis should not be made solely based on the results of the performed test but by a doctor, taking into account all clinical findings and laboratory results. The diagnosis should categorically be confirmed using various diagnostic methods.

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. A positive deviation of the results occurs in serum samples of patients who are taking Paricalcitol. 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/35.6–46.4 °F up to 48h, or frozen at -20 °C/ -4 °F for longer periods. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

7 Assay Procedure

7.1 Preparations prior to starting

All of the reagents should be held at room temperature for at least 90 minutes before use, and they should be well mixed by being inverted several times.

Reconstitution of lyophilized 25(OH)D biotin concentrate:

Add 3 ml 25(OH)D buffer to the flask with the lyophilized 25(OH)D biotin concentrate (blue color). Insert the stopper and let stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution. Add the reconstituted 25(OH)D biotin concentrate (3 ml) back to the flask with the remaining buffer. Mix the content well by inverting the flask. The diluted biotin solution is green. After production, store **protected from light** at 2–8 °C/35.6–46.4 °F.

Dilute concentrated reagents:

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

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 300 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/35.6–46.4 °F).

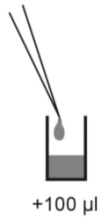
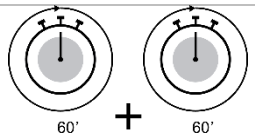
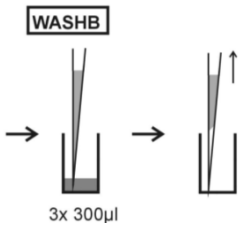
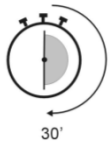
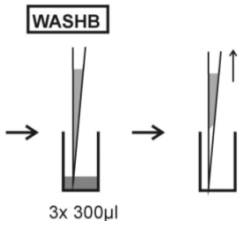
7.2 Pipetting Scheme

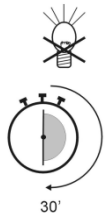
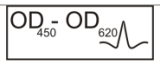
We recommend to pipette calibrators, controls and samples as follows:

	1	2	3	4...
A	Cal A	Cal E	Con 2	…
B	Cal A	Cal E	Con 2	…
C	Cal B	Cal F	P1	
D	Cal B	Cal F	P1	
E	Cal C	Cal G	P2	
F	Cal C	Cal G	P2	
G	Cal D	Con 1	P3	
H	Cal D	Con 1	P3	

Cal A: calibrator A	Cal D: calibrator D	Cal G: calibrator G	P1: patient 1
Cal B: calibrator B	Cal E: calibrator E	Con 1: control 1	P2: patient 2
Cal C: calibrator C	Cal F: calibrator F	Con 2: control 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.	
2.	Label a borosilicate glass or polypropylene test tube for each calibrator, control, and sample.	
		Calibrators, Controls & Samples
3.		Add 10 µl of each calibrator, control, and sample to the labeled test tubes. Add 400 µl of the prepared 25(OH)D biotin solution to all the test tubes. Vortex thoroughly for 10 seconds. Add 100 µL of each diluted calibrator, control, or sample to the corresponding well in duplicate.
4.		Cover the microtiter plate. Incubate for 2 hours at 18-25 °C/64.4-77 °F.
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.		Add 100 µL enzyme conjugate solution to each well within 5 minutes after the washing step.
7.		Cover the microtiter plate. Incubate for 30 minutes at 18-25 °C/64.4-77 °F.
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.		Add 100 µL TMB substrate solution to each well within 5 minutes after the washing step.
10.		Cover the microtiter plate. Incubate for 30 minutes at 18-25 °C/64.4-77 °F, protect against strong light.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate for 5 minutes at RT
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended reference filter 620 nm) within 30 minutes.

8 Calculation of results

The **quantitative evaluation** is based on a standard curve in which the optical density of the calibrators (y-axis) is plotted against the concentration in ng/ml (x-axis). A log-linear plot is recommended for evaluation. A 4-parameter logistic curve adaptation (4PL) including the “A” calibrator is required. Other algorithms are not recommended for curve adaptation. Using the curve, the 25(OH) vitamin D concentration is established from the optical density of the sample.

Alternatively, a calibration curve can be drawn on semilogarithmic graph paper. The average absorption is plotted on the y-axis against the concentration of 25(OH) vitamin D on the x-axis. Calibrator A should be in the calibration curve.

Converting the units:

Conversion of the results into mass units

ng/ml = nmol/l x 0.40

Conversion of the results into SI units

nmol/l = ng/ml x 2.5

Example of a standard curve

A new calibration curve must be prepared for each test. Note that the calibrator concentrations are specific to each batch.

Do not use this example to evaluate patient sera!

Calibrator	Concentration (ng/ml)	OD 450/620 nm
A	0.37	2.224
B	5.27	1.589
C	10.23	1.270
D	21.38	0.895
E	36.95	0.644
F	57.56	0.474
G	90.20	0.336

Example of calculation

Patient	Replikat (OD)	Mean (OD)	Result (ng/ml)	Result (nmol/l)
P 01	1.075/1.125	1.100	14.26	35.65
P 02	0.488/0.458	0.473	57.81	144.53

Samples with 25(OH)D concentrations above the measuring range must be diluted and reevaluated with a low-concentration human serum sample. The results of the diluted samples must be multiplied by the corresponding dilution factor and corrected for the low sample concentration.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

The kit contains the two controls that help validate the results obtained with each assay plate. If a control value lies outside of the acceptable range, the validity of the results should be assessed by determining if the control value deviates significantly from the averages of the laboratory series. Moreover, the following technical factors should be assessed: expiry dates of the reagents, storage conditions, pipettes, used equipment, photometer, incubation conditions, and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

Evaluation of the values

The optimum 25(OH)D concentration has not been established. There are many factors that can influence the values. They include nutrition, time of day, time of year, geographic location, exposure to sunlight, age, skin color, and genetic background. Each laboratory should establish its own range for its 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 [1, 2].

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 intoxication	> 160 ng/ml	> 400 nmol/l

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

[2] 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:	10 µl serum
Total incubation time:	3 hours at 18-25 °C/64.4-77 °F
Calibration range:	0 - 100 ng/ml (0 - 250 nmol/l), lot specific
Analytical sensitivity:	
LoB	1.51 ng/ml (3.78 nmol/l)
LoD	3.66 ng/ml (9.15 nmol/l)
LoQ	4.54 ng/ml (11.35 nmol/l)
Storage:	2–8 °C/35.6–46.4 °F in original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Normal Range

Sera of healthy donors have been investigated on ***AESKULISA®* 25OH Vitamin D** and resulted in the following distribution:

Number of Samples	Mean	Median	Highest 25(OH)D concentration	Lowest 25(OH)D concentration
120	19.14 ng/ml	18.12 ng/ml	58.02 ng/ml	<4.54 ng/ml

We also recommend that each laboratory should establish its own normal range.

10.2 Precision

Precision of test results obtained with ***AESKULISA®* 25OH Vitamin D**, REF 3810 were assessed by the determination of the intra- and inter-assay precision as well as the lot-to-lot variance by the analysis of the multiple samples of different Vitamin D concentrations.

Sample-ID	Intra Assay Precision		Inter Assay Precision		LOT to LOT Precision	
	mean (ng/ml)	CV	mean (ng/ml)	CV	mean (ng/ml)	CV
Sample 1	7.62	12.77	7.62	17.28	7.77	12.85
Sample 2	17.68	11.40	17.68	14.17	17.38	7.89
Sample 3	26.50	8.16	26.50	12.00	24.08	9.65
Sample 4	31.19	8.68	31.19	12.62	29.54	6.91
Sample 5	36.87	7.62	36.87	10.30	34.94	7.10
Sample 6	60.15	12.56	60.15	12.23	59.09	14.78

10.3 Sensitivity

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were investigated by several analyses of sample buffers and low positive samples.
The following values were determined for the ***AESKULISA®* 25OH Vitamin D**, REF 3810:

Sensitivity	25(OH)D concentration	
LoB	1.51 ng/ml	3.76 nmol/L
LoD	3.66 ng/ml	9.15 nmol/L
LoQ	4.54 ng/ml	11.35 nmol/L

10.4 Specificity

To determine the specificity of the ***AESKULISA®* 25OH Vitamin D**, REF 3810, endogenous and synthetic vitamin D metabolites were spiked into serum samples, and the following results were measured:

Cross Reactant	Spike concentration (ng/ml)	Mean % Cross Reactivity
25(OH)D_3	10.0 20.0	95 %
25(OH)D_2	10.0 20.0	109%
24,25$(OH)_2D_3$	4.0 6.0	91%
3-epi-25(OH)D_3	100	-1%
3-epi-25(OH)D_2	100	-1%
1,25-$(OH)_2D_3$	2	5%
1,25-$(OH)_2D_2$	20	84%
Vitamin D_3 (Cholecalciferol)	1000	0%
Vitamin D_2 (Ergocalciferol)	100	6%
Paricalcitol	100	17%

10.5 Linearity

Three sera covering the whole test range were diluted serially with a highly deficient serum sample. Measured and expected values of the distinct dilutions were used to calculate a linear regression. For the ***AESKULISA®* 25OH Vitamin D**, REF 3810, the linear regression of the observed concentrations in comparison to the anticipated concentrations yielded a correlation coefficient of R^2=0.99.

10.6 Method comparison

The ***AESKULISA®* 25OH Vitamin D**, REF 3810 was compared with another commercially available assay for quantitatively determining 25(OH) vitamin D. Overall, 110 samples with 25(OH) vitamin D concentrations of 6.74 ng/mL to 71.98 ng/mL were tested. A Passing-Bablok regression analysis was performed for the comparative samples:

n	Slope	95% CI	Intercept	95% CI	R^2
80	0.898	0.828 bis 0.970	0.692 ng/ml	-0.980 bis 2.553 ng/ml	0.901

11 Disposal

Please observe the relevant statutory requirements!

12 Literature

Dusso AS et al. Vitamin D. Am J Physiol Renal Physiol. 2005;289(1):F8-28.

Holick MF. The vitamin D epidemic and its health consequences. J Nutr. 2005;135(11):2739S-48S.

Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006;81(3):353-73.

Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.

Cannell JJ et al. Diagnosis and treatment of vitamin D deficiency. Expert Opin Pharmacother. 2008;9(1):107-18.

Zerwekh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr. 2008;87(4):1087S-91S.

Kennel KA et al. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc. 2010;85(8):752-7.

Wallace AM et al. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. Steroids. 2010;75(7):477-88.

Sempos CT et al. Vitamin D Standardization Program (VDSP). Vitamin D status as an international issue: national surveys and the problem of standardization. Scand J Clin Lab Invest Suppl. 2012;243:32-40.

Gröber U et al. Vitamin D: Update 2013: From rickets prophylaxis to general preventive healthcare. Dermatoendocrinol. 2013;5(3):331-47.

Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc. 2013;88(7):720-55.

Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. 2013;5(1):111-48.

Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord. 2017;18(2):153-165.

Phinney KW et al. Baseline Assessment of 25-Hydroxyvitamin D Reference Material and Proficiency Testing/External Quality Assurance Material Commutability: A Vitamin D Standardization Program Study. J AOAC Int. 2017 1;100(5):1288-1293.

Burdette CQ et al. Establishing an Accuracy Basis for the Vitamin D External Quality Assessment Scheme (DEQAS). J AOAC Int. 2017;100(5):1277-1287.

Bouillon R, Carmeliet G. Vitamin D insufficiency: Definition, diagnosis and management. Best Pract Res Clin Endocrinol Metab. 2018;32(5):669-684.

13 Regulatory Symbols

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	
UDI	- Identificatore univoco del dispositivo	- Unique Device Identifier
	- Identifiant unique de l'appareil	- Identificador único del dispositivo
	- eindeutige Produktidentifizierung	- Μοναδικό αναγνωριστικό συσκευής
	- Identificador único do dispositivo	
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	
eIFU WWW.AESKU.COM	- Rispettare le istruzioni elettroniche per l'uso	- See electronical instructions for use
	- Voir les instructions d'utilisation électronique	- Siga las instrucciones electrónicas de uso
	- Elektronische Gebrauchsanweisung beachten	- Ακολουθήστε τις ηλεκτρονικές οδηγίες χρήσης
	- Seguir as instruções electrónicas de utilização	
	- 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 (35.6-46.4°F)	- Store at 2-8°C (35.6-46.4°F)
	- Conserver à 2-8°C (35.6-46.4°F)	- Conservar a 2-8°C (35.6-46.4°F)
	- Lagerung bei 2-8°C (35.6-46.4°F)	- Φυλάσσεται στους 2-8°C (35.6-46.4°F)
	- Conservar entre 2-8°C (35.6-46.4°F)	
	- Prodotto da	- Manufactured by
	- Fabriqué par	- Fabricado por
	- Hergestellt von	- Κατασκευάζεται από
	- Fabricado por	
CON	- Controllo	- Control
	- Contrôle	- Control
	- Kontrolle	- ορός ελέγχου
	- Controlo	
CAL	- Calibratore	- Calibrator
	- Etalon	- Calibrador
	- Kalibrator	- Αντιδραστήριο βαθμονόμησης
	- Calibrador	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
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	
25(OH)D BIO 50x	- Concentrato di biotina 25-D	- 25(OH)D biotin concentrate
	- Concentré de biotine 25-D	- Concentrado de 25-D biotina
	- 25(OH)D-Biotinkonzentrat	- συμπύκνωμα 25(OH) D βιοτίνης
	- Concentrado de biotina 25-D	
25(OH)D BUF	- Tampone de 25(OH)D	- 25(OH)D buffer
	- Tampon de 25(OH)D	- Tampón de 25(OH)D
	- 25(OH)D Puffer	- Διάλυμα 25(OH)D
	- Tampão de 25(OH)D	