AESKULISA Borrelia-LIQ IgM

for use with Borrelia AESKU-LISA REF 3803

Instruction manual

Contents

1. Intended Use

AESKULISA Borrelia-LIQ IgM is a solid phase enzyme immunoassay for the quantitative and qualitative detection of IgM antibodies against Borrelia burgdorferi. The test is proposed to determine IgM antibodies in a semi-quantitative form in human serum and CSF (cerebrospinal fluid). The determination of the antibodies is performed to help in the diagnosis of acute and chronic neuroborreliosis and for the reliable determination of antibodies to Borrelia produced intrathecally.

2. Clinical Application and Principle of the Assay

The determination of intrathecally produced antibodies is an important criterion to diagnose inflammable diseases in the central nervous system. Therefore serum-CSF pairs have to be determined in parallel because the antibody content in CSF is influenced by three different factors: the antibody concentration in serum, the functionality of the blood-brain barrier and by a possible additional intrathecal antibody production. To analyse these influences it is absolutely necessary to determine the albumin and the total-IgM-concentration in serum and CSF next to the antibody determinations.

Pathogen specific Antibodies against Borrelia of the IgM class show the same CSF/serum allocation like the polyspecific total-IgM. This means that the concentration gradient from serum to CSF for total-IgM (calculated as quotient QIgMtotal) and for the specific IgM-antibodies (calculated as quotient QIgM-spez) have to be identical. The specific Antibody index (AI) calculated with these quotients describes the ratio QIgMspec and QIgMtotal.

A local synthesis of antibodies is given if the Qspec of a specific immunoglobulin class is higher as the total-immunoglobulin-quotient. If the intrathecal antibody synthesis of a patient is generated by a different origin the QlimIgMtotal has to be used for the calculation of the antibody index instead of the QIgM-total. The QlimIgMtotal describes the maximum part of total-IgM from serum in the CSF in correlation to the status of function of the blood brain barrier (expressed as Albumin CSF/Serum-quotient QAlb).

The IgM test is coated with purified OspC and Borrelia specific p41i both antigens generating primarily a high antibody response of the subclass IgM. For this reason both antigens act as specific markers for an early Borrelia infection. Additionally, RF absorbens is contained in the sample buffer. It removes specific IgG antibodies that interfere with the measurement of IgM. Moreover, RF absorbens impairs a false positive result by binding rheumatoid factors that target antigen bound IgG antibodies.

Principle of the test

Related to the dilution recommendations (chapter 7) the diluted serum/CSF samples are pipetted into the coated vials. The specific antibodies bind to the antigens on the surface of the microtiterplate. Unbound components are washed away. In a second step anti-human immunoglobulin coupled to horseradish-peroxidase are added (conjugate). These immunoglobulin bind to the antigen-antibody-complex established before. Redundant conjugate is washed away by a second washing step. The determination of bound antibodies is done by an enzymatic colour reaction which is stopped by addition of acid.. The intensity of the colour resulting is corresponding to the concentration of antibodies in the sample.

Principle of calculation:

The test is based on an solid phase enzyme immunoassay with endpoint determination. The results are given in ODs and can easily be calculated as INDEX by dividing the OD of the sample with the OD of the calibrator. The resulting values have to be filled into the Calculation program next to the values for albumin and total-IgM The Calculation program is provided separately directly from AESKU.Diagnostics.

The quotients described above are based on the following formulas:

1. QIgM = IgMCSF/IgMSerum

2. QAlb = AlbCSF/AlbSerum

3. Qspec = NDXCSF/NDXSerum x correction factor. The correction factor is necessary to consider the different dilution in serum and CSF.

4. QlimIgM = 0.93 x (radical(QAlb x QAlb + 0.000006)) – 0.0017

5. Corrective factor = volume Serum/volume CSF

3. Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: light green solution)

Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative), RF absorbens

Caution! Please do not mistake the sample buffer of Borrelia-G (yellow solution) for the sample buffer of Borrelia-M (light green solution) due to the addition of RF absorbens in the latter case!

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)

Containing: Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: colorless solution)

Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

CC Calibrator 1 vial, 1.5 ml (capped blue: yellow solution)

Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration: yellow solutions)

Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Conjugate 1 vial,15 ml IgM (capped green: green solution)

Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)

Containing: Stabilized TMB/H_2O_2

Stop Solution 1 vial, 15 ml (capped white: colorless solution)

Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells

Coating see paragraph 10.2

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware(cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multi-channel 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. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, 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 normal 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.

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.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. The blood and CSF samples have to be taken in correspondence of national laws.

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 immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
e.g. 4 ml concentrate plus 196 ml distilled water.

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 again.

Microplates:

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

7.2.

Dilution of the samples:

We recommend the following initial dilutions:
Serum:1:100
CSF:1:4

The INDEX-values (NDX) necessary for the calculation should not be higher than 4 to take care of the linearity of the measurements. This advice will be given automatically using the Calculation program. In such cases the sample has to be diluted.

7.2 Work flow

- Pipette 100µL Cut-Off calibrator, Negative-Control and diluted samples into the respec tive wells (see pipetting scheme attached)
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.
- Fill in the calculated NDX (INDEX) values into the respective column of the Calculation program.

The antibody-index values will be calculated automatically.

8. Interpretation

The evaluation of the assay was performed using a standard curve. The CSF/Serum related instructions for use is based on Index-values. These Index-values correlate to the quantitative values obtained by the standard curve. So the use of NDX values for the calculations gives similar results compared to results calculated with quantitative values using a standard curve. Therefore the Cut-Off Calibrator is pipetted twice for double determinations and the respective OD values of the samples are divided with the OD value of the calibrator.
The Calculation program automatically takes into consideration threshold values so that aberrant antibody index values are not calculated. These threshold values were established during the evaluation of the kit with specific sample panels. For confirmation of the antibody index values (AIs) serum samples were diluted to the immunoglobulin content of SCF samples and measured in parallel.
The index values have to filled into the Calculation programas described in chapter 2. In addition the respective albumin and total-IgM values have to be added next to the used dilution of the samples. The program automatically calculates the antibody index (AI). Only antibody index values will be calculated that correspond to the threshold values mentioned above.
Antibody index values higher than 1.5 have to be seen as a sign for an antibody production inside of the CNS.

9. Technical Data

Sample material:	serum/liquor
Sample volume:	100 µl diluted
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F use original vials, only
Number of determinations:	96 tests

10. Performance Data

10.1 Analytical sensitivity

Testing sample buffer 30 times on *AESKULISA Borrelia-LIQ* gave an analytical sensivity of 1.0 U/ml.

10.2 Specificity ans Sensitivity

The microplates are coated with ***purified OspC and Borrelia specific p41i .*** No crossreactivities to other autoantigens have been found. The sensitivity of the *AESKULISA* Borrelia Assays was determined to be greater than 95% in comparison to sera with known immune status. Clinically defined sera show a specificity of >96% for IgG/IgM.

10.3 Linearity

Please take into consideration that due to the heterogeneity of human antibodies some sera may show a not linear dilution behaviour.

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	449.5	470.0	95.6
	1 / 200	255.3	235.0	108.6
	1 / 400	125.1	117.5	106.4
	1 / 800	55.9	58.8	95.1
2	1 / 100	233.0	230.0	101.3
	1 / 200	113.8	1150.0	98.9
	1 / 400	63.1	57.5	110.0
	1 / 800	26.2	28.8	91.1

10.4 Precision

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

Intra-Assay		
Sample No.	Mean (U/ml)	CV (%)
1	26.0	6.7
2	151.9	4.7
3	276.5	7.7

Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)
1	27.6	6.2
2	153.0	7.4
3	284.0	5.1

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml). The Borrelia-Liq Test uses Index values for the determination of ther samples (see Chapter 2.)

11. Literature

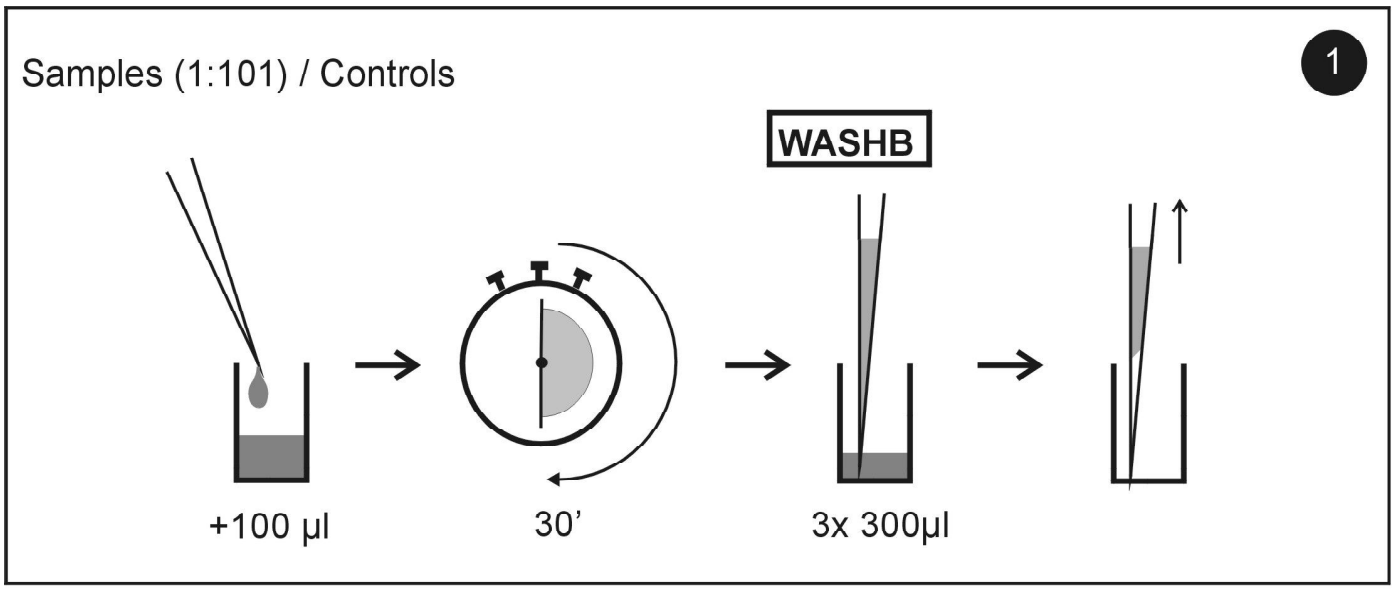
1. **Wilske B (2005).**
   Epidemiology and diagnosis of Lyme borreliosis.
   Annals of Medicine 37,8: 568-579.
2. **Stanek G, Strle F. (2003).**
   Lyme borreliosis.
   Lancet 362: 1639-1647.
3. **Bacon RM, Biggerstaff BJ, Schriefer ME, Gilmore RD Jr, Phillip MT, Steere AC, Wormser GP, Marques AR, Johnson BJ (2003).**
   Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recom binant VlsE1 or peptide antigens of Borrelia burgdorferi compared with 2 tiered testing using whole cell lysates.
   Journal of Infectious Disease 187: 1187-1199.
4. **Liang FT, Aberer E, Cinco M, Gern L, Hu CM, Lobet YN, Ruscio M, Voet PE Jr, Weynants VE, Philipp MT (2000).**
   Antigenic conservation of an immunodominant invariable region of the VlsE Lipoprotein among European pathogenic genospecies of Borrelia burgdorferi SL.
   Journal of Infectious Disease 182: 1455-1462.
5. **Marques AR., Martin DS, Philipp MT (2002).**
   Evaluation of the C6 peptide enzyme-linked immunosorbent assay for individuals vaccinated with the recombinant OspA vaccine.
   Journal of Clinical Microbiology July 2002: 2591-2593.

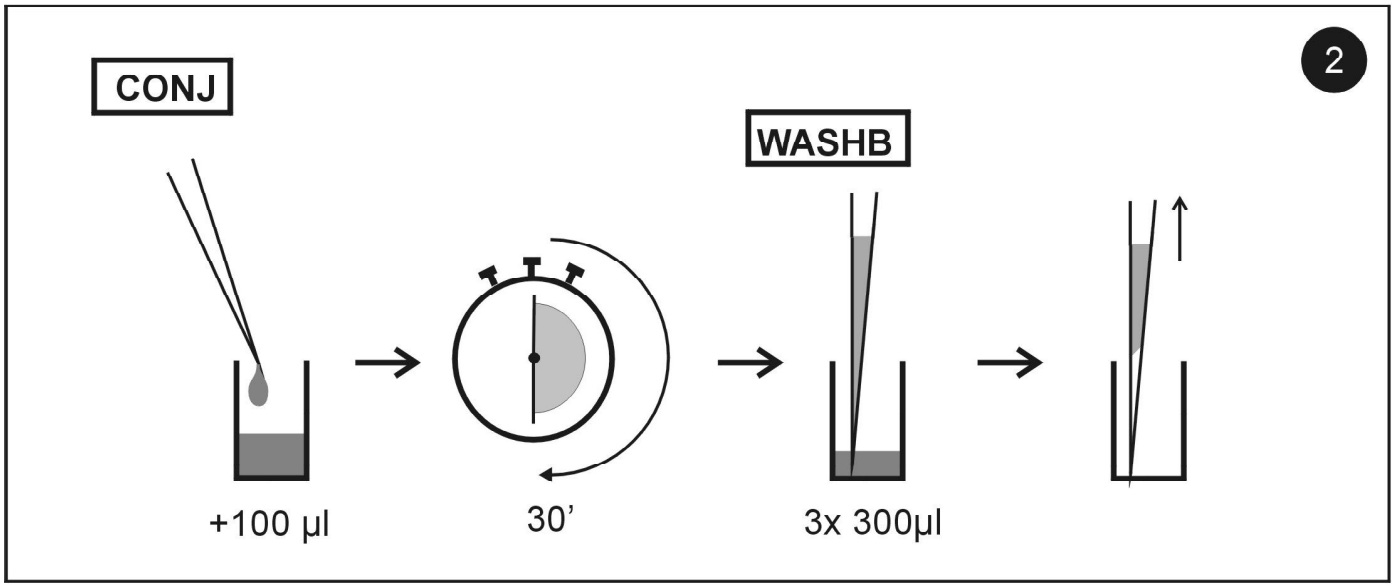
ANNEX A: Pipetting scheme

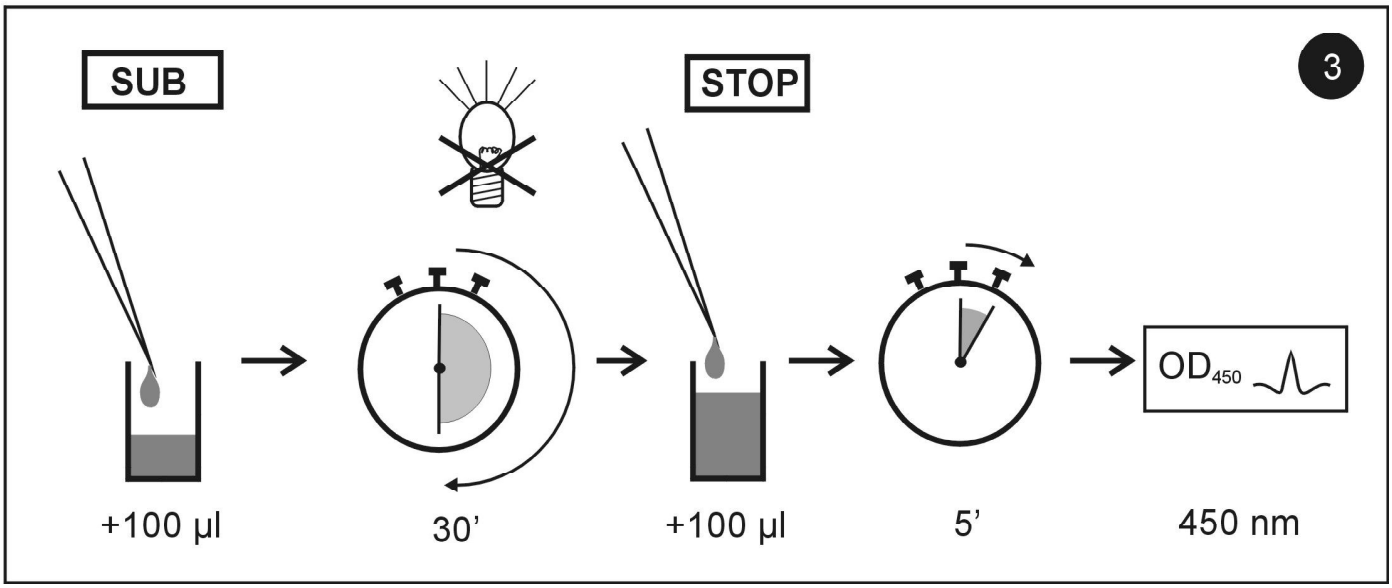
	Quantitative Auswertung											
	1	**2**	**3**	**4**	**5**	**6**						
A	NC											
B	NC											
C	CC											
D	CC											
E	P1											
F	L1											
G	P2											
H	L2											

NC: Negative Control; CC Cut off calibrator
P1: Patient 1
L2: Patient 1 CSF

Annex B: Test Procedure







Assay/Test: _____ Incubation / Inkub. : 1._____min Date/ Datum:_____

Temperature/Temperatur:_____ °F _____ °C 2._____min

Signature/Unterschrift:._____

Name:_____ 3._____min

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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IVD	♦ Diagnosi in vitro ♦ Pour diagnostic in vitro ♦ In Vitro Diagnostikum ♦ Para uso Diagnóstico in vitro	♦ For in vitro diagnostic use ♦ Para uso diagnóstico in vitro ♦ In Vitro Διαγνωστικό μέσο
REF	♦ Numero d'ordine ♦ Référence Catalogue ♦ Bestellnummer ♦ Número de catálogo	♦ Cataloge number ♦ Numéro de catálogo ♦ Αριθμός παραγγελίας
LOT	♦ Descrizione lotto ♦ Lot ♦ Chargen Bezeichnung ♦ Lote	♦ Lot ♦ Lote ♦ Χαρακτηρισμός παρτίδας
CE	♦ Conformità europea ♦ Déclaration CE de Conformité ♦ Europäische Konformität ♦ Déclaracão CE de Conformidade	♦ EC Declaration of Conformity ♦ Declaración CE de Conformidad ♦ Ευρωπαϊκή συμφωνία
96	♦ 96 determinazioni ♦ 96 tests ♦ 96 Bestimmungen ♦ 96 Testes	♦ 96 tests ♦ 96 pruebas ♦ 96 προσδιορισμοί
i	♦ Rispettare le istruzioni per l'uso ♦ Voir les instructions d'utilisation ♦ Gebrauchsanweisung beachten ♦ Ver as instrucões de uso	♦ See instructions for use ♦ Ver las instrucciones de uso ♦ Λάβετε υπόψη τις οδηγίες χρήσης
	♦ Da utilizzarsi entro ♦ Utilise avant le ♦ Verwendbar bis ♦ Utilizar antes de	♦ Use by ♦ Utilizar antes de ♦ Χρήση μέχρι
+2°C +8°C	♦ Conservare a 2-8°C ♦ Conserver à 2-8°C ♦ Lagerung bei 2-8°C ♦ Conservar entre 2-8°C	♦ Store at 2-8°C (35-46°F) ♦ Conservar a 2-8°C ♦ Φυλάσσεται στους 2-8°C
	♦ Prodotto da ♦ Fabriqué par ♦ Hergestellt von ♦ Fabricado por	♦ Manufactured by ♦ Fabricado por ♦ Κατασκευάζεται από
CO-CAL	♦ Calibratore cut-off ♦ Etalon Seuil ♦ Grenzwert Kalibrator ♦ Calibrador de cut-off	♦ Cut off Calibrator ♦ Calibrador de cut-off ♦ Οριακός ορός Αντιδραστήριο βαθμονόμησης
CON +	♦ Controllo positivo ♦ Contrôle Positif ♦ Positiv Kontrolle ♦ Controlo positivo	♦ Positive Control ♦ Control Positivo ♦ Θετικός ορός ελέγχου
CON −	♦ Controllo negativo ♦ Contrôle Négatif ♦ Negativ Kontrolle ♦ Controlo negativo	♦ Negative Control ♦ Control Negativo ♦ Αρνητικός ορός ελέγχου
CAL	♦ Calibratore ♦ Etalon ♦ Kalibrator ♦ Calibrador	♦ Calibrator ♦ Calibrador ♦ Αντιδραστήριο βαθμονόμησης
RC	♦ Recupero ♦ Corrélation ♦ Wiederfindung ♦ Recuperacão	♦ Recovery ♦ Recuperado ♦ Ανάκτηση
CONJ	♦ Coniugato ♦ Conjugé ♦ Konjugat ♦ Conjugado	♦ Conjugate ♦ Conjugado ♦ Σύζευγμα
MP	♦ Micropiastra rivestita ♦ Microplaque sensibilisée ♦ Beschichtete Mikrotiterplatte ♦ Microplaca revestida	♦ Coated microtiter plate ♦ Microplaca sensibilizada ♦ Επικαλυμμένη μικροπλάκα
PINP	♦ Piastra ad aghi rivestita ♦ Pinplate sensibilisée ♦ Beschichtete Pinplatte ♦ Pinplate revestida	♦ Coated pinplate ♦ Pinplate sensibilizada ♦ Επικαλυμμένη πλάκα Pin
WASHB 50x	♦ Tampone di lavaggio ♦ Tampon de Lavage ♦ Waschpuffer ♦ Solucão de lavagem	♦ Wash buffer ♦ Solución de lavado ♦ Ρυθμιστικό διάλυμα πλύσης
SUB	♦ Tampone substrato ♦ Substrat ♦ Substratpuffer ♦ Substrato	♦ Substrate buffer ♦ Tampón sustrato ♦ Ρυθμιστικό διάλυμα υποστρώματος
STOP	♦ Reagente bloccante ♦ Solution d'Arrêt ♦ Stopreagenz ♦ Solucão de paragem	♦ Stop solution ♦ Solución de parada ♦ Αντιδραστήριο διακοπής αντίδρασης
SB 5x	♦ Tampone campione ♦ Tampon Echantillons ♦ Probenpuffer ♦ Diluente de amostra	♦ Sample buffer ♦ Tampón Muestras ♦ Ρυθμιστικό διάλυμα δειγμάτων