AESKULISA **DF MMP-3**

REF 3167

Instruction manual

Contents

1. Intended Use

AESKULISA DF MMP-3 is a solid phase enzyme immunoassay employing two different monoclonal anti-human-MMP-3 antibodies for the quantitative determination of MMP-3 concentration in human serum.
The assay is a tool in the diagnosis of rheumatoid arthritis (RA).

2. Clinical Application and Principle of the Assay

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that is characterized primarily by the inflammation of multiple joints and leads to severe disability if disease progression cannot be stopped in an early phase. AESKULISA DF MMP-3 helps to identify RA patients who benefit from early intervention by aggressive drug therapy.
Matrix metalloproteinase-3 (MMP-3, Stromelysin-1) is a member of the matrix metalloproteinase family with a molecular mass of 52 kDa. It is expressed and secreted predominantly by connective tissue cells and plays an important role in the turnover processes of extracellular matrix (ECM). MMP-3 is able to degrade many components of the ECM including proteoglycan, fibronectin, laminin and different types of collagen. Due to this broad substrate range and its capability of activating further degrading enzymes including other matrix metalloproteinases MMP-3 plays a key role in physiological and also pathological processes of tissue remodeling.
MMP-3 is secreted as an inactive form (pro-MMP-3) and is activated through limited proteolysis by endopeptidases. Active MMP-3 can be inhibited by binding to tissue inhibitors of matrix metalloproteinases (TIMP) or α2-macroglobulin. The regulation of MMP-3 activity is crucial for avoiding matrix destruction and maintaining the physiological balance in tissue remodeling processes. According to this, elevated levels of MMP-3 have been reported in different pathological conditions like cancer, atherosclerosis, and arthritis.
Serum MMP-3 has been shown to be elevated significantly in RA patients in comparison to healthy control. Following the massive inflammation and proliferation of synovial tissue in RA patients MMP-3 expression and secretion into synovial fluid is increased. The amount of MMP-3 in the synovial fluid correlates with MMP-3 concentration in serum. Serum MMP-3 reflects the inflammation process in affected joints and is a marker for disease activity. Furthermore, high levels of serum MMP-3 have been correlated with and shown to be predictive of destructive processes in the joints of RA patients even in an early state of disease.
AESKULISA DF MMP-3 measures total MMP-3 (pro- and active MMP-3) in human serum and is a tool for risk stratification of the development of joint destruction and for control of disease activity in RA patients.

Principle of the test
AESKULISA DF MMP-3 is a sandwich ELISA using microplates coated with monoclonal anti-human MMP-3 antibody. Serum samples diluted **1:10** are incubated in the wells allowing MMP-3 present in the serum to bind to the antibody. The unbound fraction is removed by washing. Afterwards monoclonal anti-human MMP-3 antibody conjugated to horseradish peroxidase (conjugate) is incubated and reacts with the antibody-antigen complex on the microwell surface. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antibody-antigen complex and this is proportional to the initial concentration of the respective antigen (MMP-3) in the sample.

3. Kit Contents

To be reconstituted:

5x Sample Buffer	1 vial, 20 ml - 5x concentrated (capped white: yellow solution) Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative)
50x Wash Buffer	1 vial, 20 ml - 50x concentrated (capped white: green solution) Containing: Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
10x Conjugate	1 vial, 2 ml (capped blue) Containing: monoclonal anti-human MMP-3 antibody conjugated to horseradish peroxidase, bovine serum albumin (BSA)

Ready to use:

Calibrators	6 vials, 1.5 ml each 0, 25, 100, 200, 400, 800 ng/ml (capped white, color increasing with concentration: yellow solutions) Containing: Purified human MMP-3, bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Negative Control	1 vial, 1.5 ml (capped green: colorless solution) Containing: Purified human MMP-3, bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Positive Control	1 vial, 1.5 ml (capped red: yellow solution) Containing: Purified human MMP-3, bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Conjugate Buffer	1 vial, 20 ml (capped blue: blue solution) Containing: Tris, NaCl, bovine serum albumin (BSA), sodium azide <0.1% (preservative)
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 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000 ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000 ml). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper, purified water. 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 buffers and conjugate are stable at 2-8°C/35-46°F for at least 1 month. 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.
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.

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 (20-26°C/64-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

For test performance with automated systems the temperature must not exceed 26°C/78.8°F.

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.

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. 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. **EDTA and citrate plasma must not be used in this assay.**

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 at -20°C/-4°F 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).

Prepare Conjugate:

Dilute the 10x concentrated Conjugate with Conjugate Buffer (1x),
e.g. 1 ml 10x Conjugate + 9 ml Conjugate Buffer, mix well!

Prepare only as much Conjugate as you need for each run!

Diluted Conjugate has limited shelf life!

Samples:

Dilute serum samples 1:10 with sample buffer (1x)
e.g. 50 µl sample buffer + 450 µl sample buffer (1x). Mix well !

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 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B
We recommend pipetting samples and calibrators in duplicate.

- Pipette 100 µl of each patient‘s diluted serum into the designated microwells.
- Pipette 100 µl calibrators and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-26°C/64-78.8°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-26°C/64-78.8°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-26°C/64-78.8°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.

8. Quantitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in ng/ml (x-axis). For best results we recommend lin/lin coordinates and linear regression. From the OD of each sample, read the corresponding MMP-3 concentrations expressed in ng/ml.

Normal Range female
18 ng/ml - 60 ng/ml
Normal Range male
24 ng/ml - 120 ng/ml

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators MMP-3	***OD 450/620 nm***	***CV % (Variation)***
0 ng/ml	0.036	6.0
25 ng/ml	0.078	0.0
100 ng/ml	0.272	0.5
200 ng/ml	0.606	4.2
400 ng/ml	1.278	1.5
800 ng/ml	2.496	0.5

Example of calculation

Patient	**Replicate (OD)**	**Mean (OD)**	**Result (ng/ml)**
P 01	1.066/ 1.086	1.078	344.2
P 02	0.452/ 0.432	0.442	141.1

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 EU regulations.

Do not use this example for interpreting patients results!

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. If any other sample dilution than 1:10 is used the respective factor for calculation of sample values has to be included. Example: If your sample dilution is 1:20 you have to multiply the sample concentration calculated from the standard curve by factor 2.

Samples below calibrator range should be reported as < Min.

9. Technical Data

Sample material:	serum
Sample volume:	100 µl of sample diluted 1:10 with 1x sample buffer
Total incubation time:	90 minutes at 20-26°C/64-78.8°F
Calibration range:	0-800 ng/ml
Analytical sensitivity:	15 ng/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

Limit of detection

Testing sample buffer 60 times on AESKULISA DF MMP-3 and 8 low negative samples for 8 times gave a limit of detection of 15 ng/ml.

10.2 Specificity

The microplate is coated with a mouse monoclonal anti human MMP-3 antibody. No crossreactivities to other antigens have been found.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human serum there might be samples that do not follow this rule.

Sample No.	Dilution Factor	measured concentration (ng/ml)	expected concentration (ng/ml)	Recovery (%)
1	1 / 10	292.3	268.9	109
	1 / 20	149.8	146.2	102
	1 / 40	67.5	74.9	90
	1 / 80	29.9	33.7	89
2	1 / 10	651.1	649.1	100
	1 / 20	354.0	325.6	109
	1 / 40	172.5	177.0	97
	1 / 80	84.8	86.2	98

10.4 Precision

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

Intra-Assay		
Sample No.	Mean (ng/ml)	CV (%)
1	47.2	3.9
2	93.7	3.7
3	198.8	2.1
4	393.7	2.3
5	629.6	1.6

Inter-Assay		
Sample No.	Mean (ng/ml)	CV (%)
1	47.2	4.8
2	93.7	4.1
3	198.8	3.4
4	393.7	4.0
5	629.6	3.5

10.5 Calibration

Due to the lack of an international standard the AESKULISA DF MMP-3 is calibrated against defined amounts of purified human MMP-3. The results are expressed in ng/ml.

10.6 Recovery

Recovery was determined by spiking various amounts of human MMP-3 into human serum. Mean recoveries are as follows:

Serum samples	Average % recovery
Sample 1	104.5
Sample 2	105.0
Sample 3	101.4

11. Literature

1. **Y. Okada et al.: J. Biol.Chem., 261, 14245-14255, 1986.**
2. **K. Obata et al.: Clin. Chim. Acta, 211, 59-72, 1992.**
3. **J. Martel-Peletier et al.: Lab. Invest., 70, 807-815, 1994.**
4. **S. Sasaki et al.: Clin. Rheum., 13, 228-233, 1994.**
5. **Y. Yoshihara et al.: Arthritis. Rheum., 38, 969-975, 1995.**
6. **Y. Yoshihara et al.: Arthritis. Rheum., 38, 969-975, 1995.**

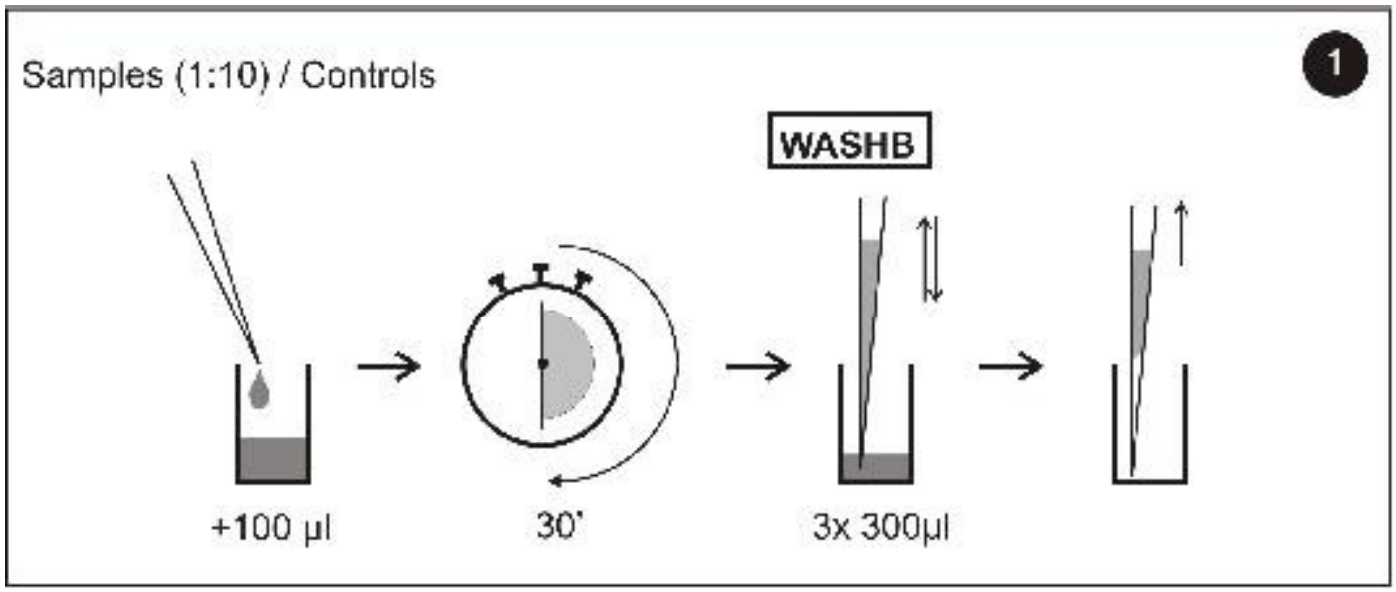
ANNEX A: Pipetting scheme

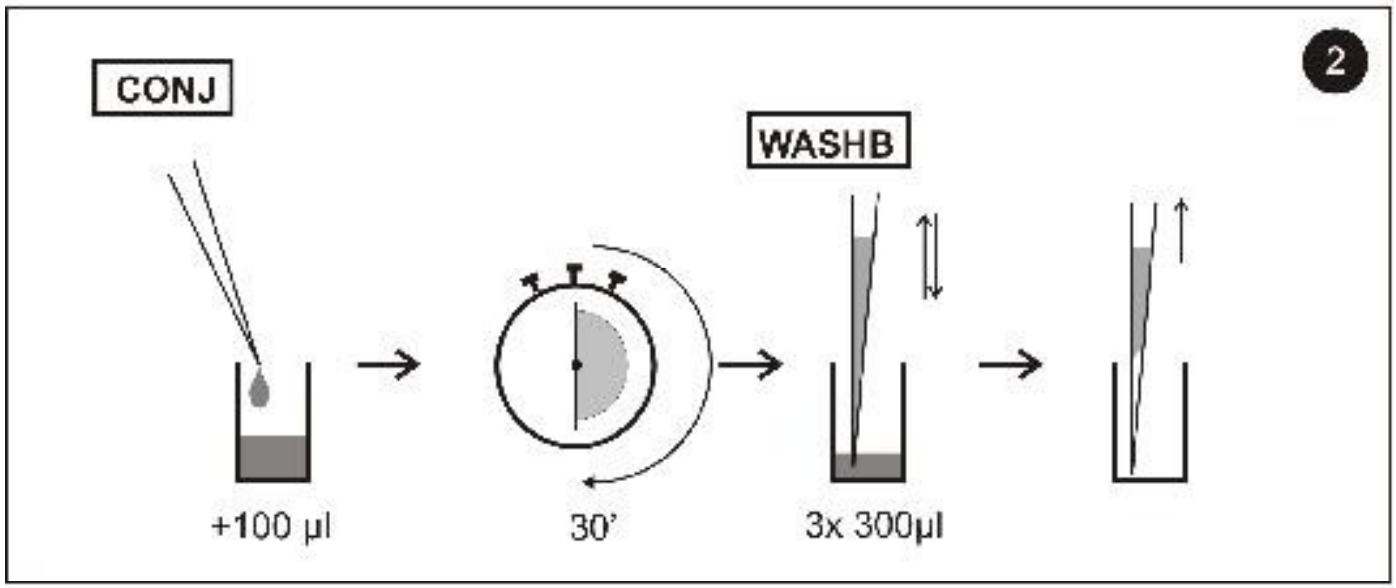
We suggest pipetting calibrators, controls and samples as follows:
For **quantitative interpretation** always use calibrators to establish a standard curve.

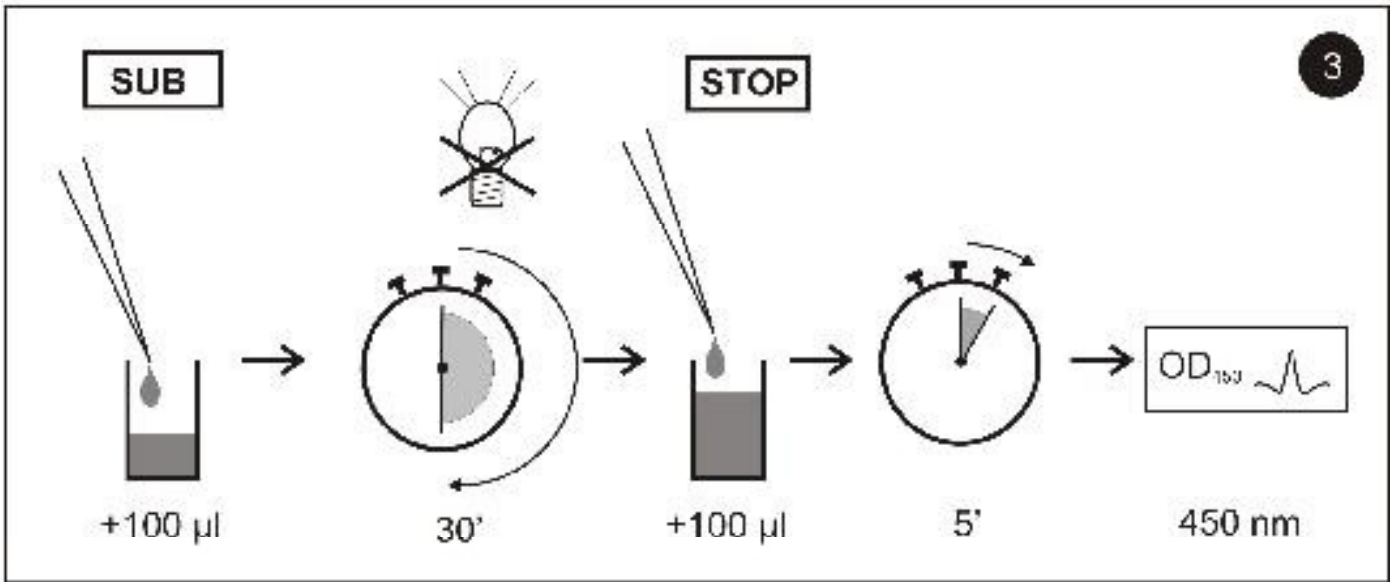
	for **quantitative interpretation** use calibrators to establish a standard curve					
	1	**2**	**3**	**4**	**5**	**6**
A	CalA	CalE	P1			
B	CalA	CalE	P1			
C	CalB	CalF	P2			
D	CalB	CalF	P2			
E	CalC	PC	P3			
F	CalC	PC	P3			
G	CalD	NC	...			
H	CalD	NC	...			

CalA: calibrator A, CalB: calibrator B, CalC: calibrator C, CalD: calibrator D, CalE: calibrator E, CalF: calibrator F
PC: positive control
NC: negative control
P1: patient 1
P2: patient 2
P3: patient 3

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
CB	♦ Tampone Calibratore ♦ Tampon Calibrateur ♦ Kalibrator Puffer ♦ Calibrador Tampão	♦ Calibrator Buffer ♦ Tampón calibrador ♦ Τυπικό ρυθμιστικό διάλυμα
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