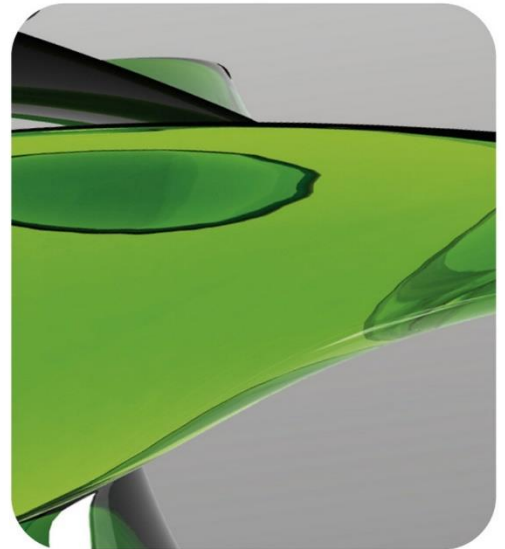




**AESKU**.DIAGNOSTICS  
THE DIAGNOSTIC TOOL THAT WORKS



**AESKULISA<sup>®</sup>**

THE DIAGNOSTIC TOOL THAT WORKS

# INSTRUCTION MANUAL

**AESKULISA ASCA-G**

Ref 3508







Product Ref.	3508
Product Desc.	ASCA-G
Manual Rev. No.	004: 2017-10-19

# Instruction Manual

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## 1 Intended Use

**AESKULISA ASCA-G** is a solid phase enzyme immunoassay (ELISA) employing highly purified mannan for the quantitative and qualitative detection of IgG anti-Saccharomyces cerevisiae antibodies (ASCA) in human serum. ASCA recognize specific mannan, a component of the outer cell wall of yeast. The assay is highly specific and sensitive for Crohn`s disease.

## 2 Clinical Application and Principle of the Assay

Crohn`s disease is one of the two major Inflammatory Bowel Diseases (IBD). IBD is an umbrella term, that covers both primary disorders causing inflammation or ulceration in the small and large intestine, Crohn`s disease and ulcerative colitis. Crohn`s disease affects both, the small bowel and the colon, unlike ulcerative colitis which is restricted to the colon only. The etiology is not revealed yet, although a genetic and infectious background for the disease is under discussion. Colonoscopy and ileoscopy are the established tools of diagnosis, no serology was available so far. Though Crohn`s disease and ulcerative colitis share several symptoms the course of the diseases, its complications and its management are different, especially when it comes down to surgery. Consequently the differential diagnosis of both diseases is crucial prior to treatment. Aggravating, about 5-10% of the patients can not be distinguished clearly by existing available diagnostic methodologies and are referred to as indeterminate colitis.

ASCA have been found to be specific markers for Crohn`s disease. They have been reported for these patients with a frequency of 68%. The identification of the target antigen mannan, a mannose-rich carbohydrate antigen of the outer cell wall of yeast, enabled the detection of ASCA by enzyme immunoassay.

Being the first available highly specific serological marker, ASCA may become an important tool for the difficult task of diagnosing IBD. Moreover its high positive predictive value offers the only possibility of a convenient and reliable screening and monitoring of risk groups.

### Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient`s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. 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 antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

### 3 Kit Contents

<b>TO BE RECONSTITUTED</b>				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
<b>READY TO USE</b>				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H <sub>2</sub> O <sub>2</sub> )
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				
<b>MATERIALS REQUIRED, BUT NOT PROVIDED</b>				
Microtiter plate reader 450 nm reading filter and recommended 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 (100-1000µl). 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 solutions are stable at 4°C/39°F for 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 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 ( $\text{NaN}_3$ ) as a preservative.  $\text{NaN}_3$  may be toxic if ingested or adsorbed by skin or eyes.  $\text{NaN}_3$  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 biological source material used for some reagents of this kit 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 these 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 (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at 30°C/86°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.

**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

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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, respectively stored tightly closed at 2-8°C/35-46°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

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### 7.1 Preparations prior to starting

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).

To avoid mistakes we suggest to mark the cap of the different calibrators.

#### **Samples:**

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. 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 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For <i>QUANTITATIVE</i> interpretation					For <i>QUALITATIVE</i> interpretation				
	1	2	3	4...		1	2	3	4...
<b>A</b>	Cal A	Cal E	P1		<b>A</b>	NC	P2		
<b>B</b>	Cal A	Cal E	P1		<b>B</b>	NC	P2		
<b>C</b>	Cal B	Cal F	P2		<b>C</b>	CC	P3		
<b>D</b>	Cal B	Cal F	P2		<b>D</b>	CC	P3		
<b>E</b>	Cal C	PC	P3		<b>E</b>	PC	...		
<b>F</b>	Cal C	PC	P3		<b>F</b>	PC	...		
<b>G</b>	Cal D	NC	...		<b>G</b>	P1	...		
<b>H</b>	Cal D	NC	...		<b>H</b>	P1	...		

CalA: calibrator A

CalB: calibrator B

CalC: calibrator C

CalD: calibrator D

CalE: calibrator E

CalF: calibrator F

PC: positive control

NC: negative control


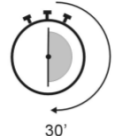
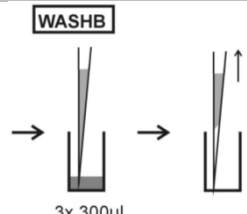
CC: cut-off calibrator

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.
2.	Use the following steps in accordance with quantitative/ qualitative interpretation results desired:
<b>CONTROLS &amp; SAMPLES</b>	
3.	 <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:</p> <ol style="list-style-type: none"> <li>Calibrators (CAL.A to CAL.F) for <i>QUANTITATIVE</i> or</li> <li>Cut-off Calibrator (CC) for <i>QUALITATIVE</i> interp.</li> </ol> <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> <li>Negative control (NC) and Positive control (PC), and</li> <li>Patients diluted serum (P1, P2...)</li> </ul>
4.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
5.	 <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>





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### CONJUGATE

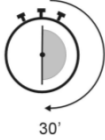
6.

**CONJ**



Pipette 100 µl conjugate into each well.

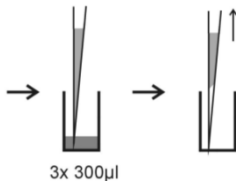
7.



Incubate for 30 minutes at 20-32°C/68-89.6°F.

8.

**WASHB**



Wash 3x with 300 µl washing buffer (diluted 1:50).

### SUBSTRATE

9.

**SUB**



Pipette 100 µl TMB substrate into each well.

10.

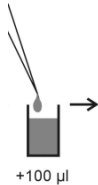


Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.

### STOP

11.

**STOP**



Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.

12.

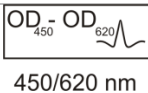


Incubate 5 minutes minimum.

13.

Agitate plate carefully for 5 sec.

14.



Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

## 8 Quantitative and Qualitative 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 U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

### Example of a standard curve

**Do NOT use this example for interpreting patient's result**

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 U/ml	0.032	2.8
3 U/ml	0.152	2.6
10 U/ml	0.281	1.2
30 U/ml	0.646	2.4
100 U/ml	1.214	1.7
300 U/ml	2.104	1.6

### Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.904/0.937	0.921	58.3
P 02	0.564/0.551	0.558	25.9

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

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.

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

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, 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.

For **qualitative interpretation** read the optical density of the cut-off calibrator and the patient samples. Compare patient's OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

<b>Negative:</b>	OD patient	<	0.8 x OD cut-off
<b>Equivocal:</b>	0.8 x OD cut-off	≤	OD patient ≤ 1.2 x OD cut-off
<b>Positive:</b>	OD patient	>	1.2 x OD cut-off

## 9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	4.13 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

## 10 Performance Data

### 10.1 Normal Range

Sera of healthy donors have been investigated on AESKULISA ASCA-G and resulted in the following distribution:

Number of Samples	negative	borderline	positive
298	195 (65.4 %)	24 (8.1 %)	69 (23.2%)

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

### 10.2 Precision

Precision of test results obtained with AESKULISA ASCA-G, REF 3508 were assessed by the determination of the intra- and inter assay precision as well as the lot-to-lot variance by the analysis of multiple samples of different antibody activities.

Sample ID	Intra Assay Precision		Inter Assay Precision		LOT to LOT Precision	
	Mean (U/ml)	CV	Mean (U/ml)	CV	Mean (U/ml)	CV
Sample 1	11.0	17.4%	9.7	15.0%	9.7	6.6%
Sample 2	36.8	8.9%	35.0	11.8%	35.0	5.7%
Sample 3	57.7	5.0%	57.4	9.9%	57.4	4.2%
Sample 4	93.3	9.3%	92.1	9.1%	92.1	5.1%
Sample 5	168.8	9.7%	168.4	9.6%	168.4	4.1%
Sample 6	180.6	9.2%	179.3	9.6%	179.3	4.0%

### 10.3 Sensitivity and Specificity

#### Analytical sensitivity

The analytical sensitivity has been assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection.

For AESKULISA ASCA-G, REF 3508 a **LoD of 4.13 U/ml** has been determined.

## 10.4 Linearity

Three sera covering the whole test range were diluted serially with a negative serum sample. Measured and expected values of the distinct dilutions were used to calculate a linear regression. According to results of linearity testing a measurable range of 3 - 300 IU/ml was determined for AESKULISA ASCA-G.

## 10.5 Calibration

Due to the lack of international reference calibration AESKULISA ASCA-G is calibrated in arbitrary units (U/ml).

## 11 Disposal

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Please observe the relevant statutory requirements!

## 12 Literature

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*Anti-Saccharomyces cerevisiae antibodies in inflammatory bowel disease: a family study.*

Scand J Gastroenterol 36: 196-201.

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Clin Diagn Lab Immunol 3: 219-226.

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Gut 33: 1071-1075.

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Am. J. Epidemiol. 149(10): 916-924.

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*Clinical value of the detection of antibodies in the serum for diagnosis and treatment of inflammatory bowel disease.*

Gastroenterol. 115: 1006-1022

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Int. J. Colorectal Dis. 19: 319-24

**10. Lawrance IC, Murray K, Hall A, Sung JJ, Leong R. (2004)**

*A prospective comparative study of ASCA and pANCA in Chinese and Caucasian IBD patients.*

Am. J. Gastroenterol. 99: 2186-94

**11. Damoiseaux JG, Bouten B, Linders AM, Austen J, Roozendaal C, Russel MG, Forget PP, Tervaert JW. (2002)**

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*Anti-Saccharomyces cerevisiae antibodies (ASCA) and autoimmune liver diseases.*

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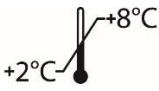

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*Familial expression of anti-Saccharomyces cerevisiae mannan antibodies in affected and unaffected relatives of patients with Crohn's disease.*

Gut 46: 58 - 63.

**14. Lothar Thomas:** Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books

**15. CLSI Guideline GP44-A4:** Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

<b>IVD</b>	- 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
<b>REF</b>	° Numero d'ordine ° Référence Catalogue ° Bestellnummer ° Número de catálogo	° Catalogue number ° Numéro de catálogo ° Αριθμός παραγγελίας
<b>LOT</b>	° Descrizione lotto ° Lot ° Chargen Bezeichnung ° Lote	° Lot ° Lote ° Χαρακτηρισμός παρτίδας
<b>CE</b>	° Conformità europea ° Déclaration CE de Conformité ° Europäische Konformität ° Declaração CE de Conformidade	° EC Declaration of Conformity ° Declaración CE de Conformidad ° Ευρωπαϊκή συμφωνία
	° 96 determinazioni ° 96 tests ° 96 Bestimmungen ° 96 Testes	° 96 tests ° 96 pruebas ° 96 προσδιορισμοί
	° Rispettare le istruzioni per l'uso ° Voir les instructions d'utilisation ° Gebrauchsanweisung beachten ° Ver as instruções 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
	° 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
<b>CO-CAL</b>	° Calibratore cut-off ° Etalon Seuil ° Grenzwert Kalibrator ° Calibrador de cut-off	° Cut off Calibrator ° Calibrador de cut-off ° Οριακός ορός Αντιδραστήριο βαθμονόμησης
<b>CON +</b>	° Controllo positivo ° Contrôle Positif ° Positiv Kontrolle ° Controlo positivo	° Positive Control ° Control Positivo ° Θετικός ορός ελέγχου
<b>CON -</b>	° Controllo negativo ° Contrôle Négatif ° Negativ Kontrolle ° Controlo negativo	° Negative Control ° Control Negativo ° Αρνητικός ορός ελέγχου
<b>CAL</b>	° Calibratore ° Etalon ° Kalibrator ° Calibrador	° Calibrator ° Calibrador ° Αντιδραστήριο βαθμονόμησης
<b>RC</b>	° Recupero ° Corrélation ° Wiederfindung ° Recuperação	° Recovery ° Recuperado ° Ανάκτηση
<b>CONJ</b>	° Coniugato ° Conjugé ° Konjugat ° Conjugado	° Conjugate ° Conjugado ° Σύζευγμα
<b>MP</b>	° Micropiastra rivestita ° Microplaque sensibilisée ° Beschichtete Mikrotiterplatte ° Microplaca revestida	° Coated microtiter plate ° Microplaca sensibilizada ° Επικαλυμμένη μικροπλάκα
<b>WASHB 50x</b>	° Tampone di lavaggio ° Tampon de Lavage ° Waschpuffer ° Solução de lavagem	° Wash buffer ° Solución de lavado ° Ρυθμιστικό διάλυμα πλύσης
<b>SUB</b>	° Tampone substrato ° Substrat ° Substratpuffer ° Substrato	° Substrate buffer ° Tampón sustrato ° Ρυθμιστικό διάλυμα υποστρώματος
<b>STOP</b>	° Reagente bloccante ° Solution d'Arrêt ° Stopreagenz ° Solução de paragem	° Stop solution ° Solución de parada ° Αντιδραστήριο διακοπής αντίδρασης
<b>SB 5x</b>	° Tampone campione ° Tampon Echantillons ° Probenpuffer ° Diluente de amostra	° Sample buffer ° Tampón Muestras ° Ρυθμιστικό διάλυμα δειγμάτων