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# AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

# INSTRUCTION MANUAL

## *AESKULISA®* Ethanolamine-GM

***Ref 3209***

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# Instruction Manual

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# 1 Intended Use

***AESKULISA®*** **Ethanolamin-GM** is a solid phase enzyme immunoassay with highly purified phosphatidyl-ethanolamin plus native human ß2-glycoprotein I for the quantitative and qualitative detection of IgG and/or IgM antibodies against phosphatidyl-ethanolamin in human serum. These antibodies recognize specific epitopes on a complex composed out of phosphatidyl-ethanolamin and ß2-glycoprotein I.

The assay is an aid in the diagnosis and risk estimation of thrombosis in patients with systemic lupus erythematosus and APS.

# 2 Clinical Application and Principle of the Assay

Antibodies against phosphatidyl-ethanolamin, an phospholipid derived from glycerol, belong to the group of anti-phospholipid antibodies specific for phospholipids such as cardiolipin, phosphatidyl- serine, -inositol, -choline, sphingomyelin and phosphatidic acid. Phospholipids are components of biological membranes.

Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The occurrence of anti-phospholipid antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-phospholipid antibodies in patients with no other autoimmune diseases characterize the primary APS.

Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarction). The exact mechanisms by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

Reports of anti-phosphatidyl-ethanolamin antibodies with similar or identical pathogenic associations as those described for anti-cardiolipin and anti-phosphatidylserine have repeatedly been described in the literature. PE is a zwitterionic phospholipid present in both internal and external sides of cell mebranes, thought to be involved in the development of thrombotic events via its role in the protein C pathway and the inactivation of factor Va by activated protein C. Antibodies directed to PE could play an important role in the pathogenesis of the APS by inhibiting activated protein C. In some instances, anti-PE antibodies are the sole antiphospholipid antibodies observed thus being an important diagnostic tool in addition to cardiolipin antibodies.

**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

| ***TO BE RECONSTITUTED*** | | | | |
|---|---|---|---|---|
| Item | Quantity | Cap color | Solution color | Description / Contents |
| Sample Buffer (5x) | 1 x 20ml | White | Yellow | 5 x concentrated<br>Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative) |
| Wash Buffer (50x) | 1 x 20ml | White | Green | 50 x concentrated<br>Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative) |
| ***READY TO USE*** | | | | |
| Item | Quantity | Cap color | Solution color | Description / Contents |
| Negative Control | 1 x 1.5ml | Green | Colorless | Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative) |
| Positive Control | 1 x 1.5ml | Red | Yellow | Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative) |
| Cut-off Calibrator | 1 x 1.5ml | Blue | Yellow | Human serum (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. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative) |
| Conjugate, IgG<br>IgM | 1 x 15ml<br>1 x 15ml | Blue<br>Green | Blue<br>Green | Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA) |
| 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 | 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), 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. Once prepared, reconstituted solutions are stable at 2-8 °C/35.6-46.4 °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 ($NaN_3$) 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 (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

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/35.6-46.4 °F up to 48 h, 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 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/35.6-46.4 °F).

## 7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

**NOTE: If IgG and IgM are determined in parallel, calibrators, controls and samples have to be done twice, for each subclass separately.**

For *QUANTITATIVE* interpretation

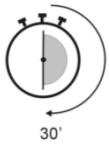
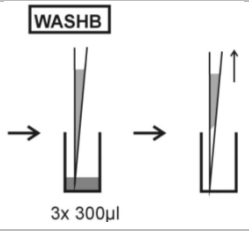
| | 1 | 2 | 3 | 4... |
|---|---|---|---|---|
| **A** | Cal A | Cal E | P1 | |
| **B** | Cal A | Cal E | P1 | |
| **C** | Cal B | Cal F | P2 | |
| **D** | Cal B | Cal F | P2 | |
| **E** | Cal C | PC | P3 | |
| **F** | Cal C | PC | P3 | |
| **G** | Cal D | NC | … | |
| **H** | Cal D | NC | … | |

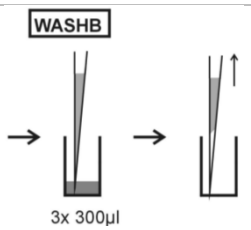
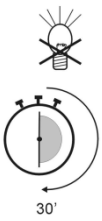
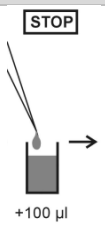
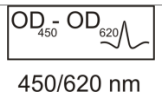
For *QUALITATIVE* interpretation

| | 1 | 2 | 3 | 4... |
|---|---|---|---|---|
| **A** | NC | P2 | | |
| **B** | NC | P2 | | |
| **C** | CC | P3 | | |
| **D** | CC | P3 | | |
| **E** | PC | … | | |
| **F** | PC | … | | |
| **G** | P1 | … | | |
| **H** | 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: |
| | CONTROLS & SAMPLES |
| 3. |  Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:<br>a. Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* or<br>b. Cut-off Calibrator (CC) for *QUALITATIVE* interp.<br>and 100 µl of each of the following:<br>• Negative control (NC) and Positive control (PC), and<br>• Patients diluted serum (P1, P2...) |
| 4. |  Incubate for 30 minutes at 20-32 °C/68-89.6 °F. |
| 5. |  Wash 3x with 300 µl washing buffer (diluted 1:50). |

| | | |
|---|---|---|
| | CONJUGATE | |
| 6. |  | Pipette 100 µl conjugate into each well. |
| 7. |  | Incubate for 30 minutes at 20-32 °C/68-89.6 °F. |
| 8. |  | Wash 3x with 300 µl washing buffer (diluted 1:50). |
| | SUBSTRATE | |
| 9. |  | 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. |  | 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/M | OD 450/620 nm | CV % (Variation) |
|---|---|---|
| 0 U/ml | 0.039 | 3.2 |
| 3 U/ml | 0.172 | 5.4 |
| 10 U/ml | 0.330 | 3.2 |
| 30 U/ml | 0.663 | 0.6 |
| 100 U/ml | 1.302 | 2.0 |
| 300 U/ml | 2.115 | 0.6 |

***Example of calculation***

| Patient | Replicate (OD) | Mean (OD) | Result (U/ml) |
|---|---|---|---|
| P 01 | 0.917/0.910 | 0.914 | 52.0 |
| P 02 | 0.443/0.454 | 0.449 | 15.8 |

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.

| | | | | | | |
|---|---|---|---|---|---|---|
| **Negative:** | | **OD patient** | **<** | **0.8 x OD cut-off** | | |
| **Equivocal:** | **0.8 x** | **OD cut-off** | **≤** | **OD patient** | **≤** | **1.2 x OD cut-off** |
| **Positive:** | | **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: | 1.0 U/ml |
| Storage: | at 2-8 °C/35.6-46.4 °F use original vials only. |
| Number of determinations: | 96 tests |

# 10 Performance Data

## 10.1 Analytical sensitivity

Testing sample buffer 30 times on ***AESKULISA®*** **Ethanolamine-GM** gave an analytical sensitivity of 1.0 U/ml.

## 10.2 Specificity and sensitivity

The microplate is coated with **Phosphatidyl-Ethanolamin** and native human **ß2-Glycoprotein I**. No crossreactivities to other autoantigens 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 autoantibodies there might be samples that do not follow this rule.

| Sample No. | Dilution Factor | Measured (U/ml) | Expected (U/ml) | Recovery (%) |
|---|---|---|---|---|
| 1 | 1 / 100 | 123.0 | 125.0 | 98.4 |
| | 1 / 200 | 61.3 | 62.5 | 98.1 |
| | 1 / 400 | 29.7 | 31.3 | 94.9 |
| | 1 / 800 | 15.2 | 15.6 | 97.4 |
| 2 | 1 / 100 | 81.4 | 80.0 | 101.8 |
| | 1 / 200 | 42.8 | 40.0 | 107.0 |
| | 1 / 400 | 21.4 | 20.0 | 107.0 |
| | 1 / 800 | 9.8 | 10.0 | 98.0 |

## 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 | 132.0 | 5.3 |
| 2 | 84.0 | 3.7 |
| 3 | 35.0 | 2.5 |

| Inter-assay | | |
|---|---|---|
| **Sample No.** | **Mean (U/ml)** | **CV (%)** |
| 1 | 128.0 | 4.8 |
| 2 | 82.0 | 3.3 |
| 3 | 33.0 | 2.7 |

## 10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

# 11 Literature

**Boey, M.L., Colaco, C.B., Gharavi, A.E., et al. (1983)**. Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. Br. Med. J. 287: 1021-1023.

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**Wöhrle R, Matthias T, von Landenberg P, Oppermann M, Helmke K, Förger F (2000).** Clinical relevance of antibodies against different phospholipids. Journal of Autoimmunity 15: A60.

**E. Balada, J. Ordi-Ros, F. Paredes, J. Villarreal, M. Mauri, M. Vilardell-Tarrés (2001).** Anti-phosphatidylethanolamine antibodies contribute to the diagnosis of antiphospholipid syndrome in patients with systemic lupus erythematosus. Scand J Rheumatol 30: 235-241.

**J.A. Mcintyre, D.R. Wagenknecht (2000)**. Anti-phosphatidylethanolamine (aPE) antibodies: a survey. J Autoimmun 15(2):185-93.

# 12 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 | |
| CO-CAL | - Calibratore cut-off | - Cut off Calibrator |
| | - Etalon Seuil | - Calibrador de cut-off |
| | - Grenzwert Kalibrator | - Οριακός ορός Αντιδραστήριο βαθμονόμησης |
| | - Calibrador de cut-off | |
| CON + | - Controllo positivo | - Positive Control |
| | - Contrôle Positif | - Control Positivo |
| | - Positiv Kontrolle | - Θετικός ορός ελέγχου |
| | - Controlo positivo | |
| CON − | - Controllo negativo | - Negative Control |
| | - Contrôle Négatif | - Control Negativo |
| | - Negativ Kontrolle | - Αρνητικός ορός ελέγχου |
| | - Controlo negativo | |
| 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 | |
| SB 5x | - Tampone campione | - Sample buffer |
| | - Tampon Echantillons | - Tampón Muestras |
| | - Probenpuffer | - Ρυθμιστικό διάλυμα δειγμάτων |
| | - Diluente de amostra | |