



REF 10901

Protein C



Instruction Manual

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| | | |
|---|-----------------|------------------|
|  | Product Ref. | 10901 |
| | Product Desc. | Protein C |
| | Manual Rev. No. | 001 : 2014-12-18 |

1 Intended Use

Protein C is a solid phase enzyme immunoassay for the quantitative determination of Protein C in citrated human plasma. The determination of Protein C aids in the risk estimation of thrombosis.

2 Clinical Application and Principle of the Assay

Protein C is a vitamin K-dependent inactive zymogen of a serine protease that is mainly synthesized by hepatocytes in the liver. It has a molecular weight of 62 kDa and is present at a concentration of 4 µg/ml in the plasma. Activated Protein C (aPC) is a key component of the Protein C anticoagulant system that is activated by the binding of thrombin to the endothelial transmembrane receptor thrombomodulin. The complex of thrombin and thrombomodulin activates Protein C and the activated Protein C in turn forms a complex with its cofactor Protein S that has a high affinity to phospholipid membranes. This is of physiological importance since aPC inactivates preferentially the membrane-bound coagulation factors Va and VIIIa. Additionally, activated Protein C possesses profibrinolytic activity by inhibiting plasmin activator inhibitor-1 (PAI-1). Protein C deficiency may be inherited or acquired and is associated with a variably increased risk of thrombosis. The prevalence of Protein C deficiency has been estimated to be up to one case per 300 in the general population. Nearly 50-80 % of individuals with inherited Protein C deficiency will experience a thrombotic event before the age of 30-45. Patients with a homozygous Protein C deficiency may suffer from neonatal purpura fulminans or massive venous thrombosis. Acquired Protein C deficiency is often associated with liver disease, surgery, oral anticoagulant therapy, antiphospholipid syndrome, etc. Protein C deficiency is classified in two states. Type I deficiency is a reduction in the level of Protein C. Type II deficiency is characterized by a reduced Protein C activity, with normal antigen level. To determine the type of defect, the laboratory diagnosis of Protein C may require both antigen levels and functional determination.

Principle of the test

The Protein C is a sandwich ELISA using microplates coated with a capture antibody specific for human Protein C. 1:51 diluted patient plasma is incubated in the wells allowing Protein C present in the plasma to bind to the antibody. The unbound fraction is removed by washing. Afterwards anti-human Protein C detection antibody conjugated to horseradish peroxidase (conjugate) is incubated and reacts with the antigen-antibody complex on the microwell surface. Following incubation, unbound conjugate is washed off. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is measured in optical density units with a spectrophotometer at 450 nm. Using a curve prepared from the Reference Plasma provided with the kit, the Protein C antigen relative percent concentration in patient plasma can be determined.

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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 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) |
| Reference Plasma | 3 x 0,4ml | White | | Containing: lyophilized human plasma |
| Control N | 3 x 0,2ml | White | | Containing: lyophilized normal human plasma |
| Control D | 3 x 0,2ml | White | | Containing: lyophilized deficient human plasma |
| READY TO USE | | | | |
| Item | Quantity | Cap color | Solution color | Description / Contents |
| Conjugate, IgG | 1 x 15ml | Blue | Blue | Containing: anti-human Protein C antibody conjugated to horseradish peroxidase, bovine serum albumin (BSA) |
| TMB Substrate | 1 x 15ml | Black | Colorless | Containing: Stabilized TMB/H ₂ O ₂ |
| Stop Solution | 1 x 15ml | White | Colorless | Containing: 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/35-46°F, in their original containers. Once prepared, reconstituted solutions except for the Reference Plasma and the Controls are stable for 1 month at 4°C/39°F. After reconstitution the Reference Plasma and the Controls are stable for 8 hours when stored at 2-8°C/35-46°F. 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.

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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 ! 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.

The Reference Plasma and the Controls included in this kit have 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 Reference Plasma, Controls and patient samples as if capable of transmitting infectious diseases and 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 ($20\text{-}26^\circ\text{C}/68\text{-}78.8^\circ\text{F}$) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at $23^\circ\text{C}/73.4^\circ\text{F}$ for automated systems.

Never expose components to higher temperature than $37^\circ\text{C}/98.6^\circ\text{F}$.

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.

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6 Sample Collection, Handling and Storage

Use preferentially plasma samples freshly collected with 3.2% or 3.8% sodium citrate as an anticoagulant. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Blood samples should be collected in clean, dry and empty tubes. After centrifugation, the plasma samples should be used immediately, otherwise stored tightly closed at 2-8°C/35-46°F up to eight hours, 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).

Reference Plasma:

Reconstitute Reference Plasma by adding 0.4 ml distilled water and shake gently. Allow the reconstituted plasma to stand for 10 minutes at room temperature before use. The Reference Plasma is stable for 8 hours when stored at 2-8°C/35-46°F.

Controls:

Reconstitute Control N and Control D by adding 0.2 ml distilled water and shake gently. Allow the reconstituted Controls to stand for 10 minutes at room temperature before use. The Controls are stable for 8 hours when stored at 2-8°C/35-46°F.

Predilution of the Reference Plasma:

Prepare a 1:2 dilution of reconstituted reference plasma in prediluted sample buffer (1x) and mix well, e.g. 100 µl sample buffer + 100 µl plasma.

Preparation of the reference curve:

The dilution set is prepared by using the prediluted Reference Plasma.

| Volume Reference Plasma | Volume Sample Buffer | Reference Level |
|-------------------------|----------------------|-----------------|
| 60 µl | 1000 µl | 150 % |
| 40 µl | 1000 µl | 100 % |
| 30 µl | 1000 µl | 75 % |
| 20 µl | 1000 µl | 50 % |
| 10 µl | 1000 µl | 25 % |
| 10 µl | 2000 µl | 12.5 % |

Dilution of the Samples and Controls:

Add 20 µl plasma to 1000 µl sample buffer (1x) and 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).

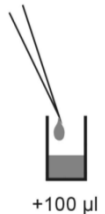
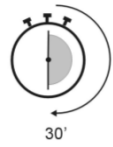
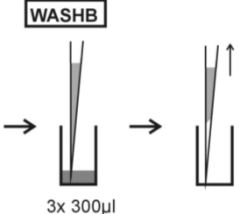
7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

| | 1 | 2 | 3 | 4... | |
|----------|-----|------|-----|------|--|
| A | 150 | 25 | P1 | | |
| B | 150 | 25 | P1 | | |
| C | 100 | 12.5 | P2 | | |
| D | 100 | 12.5 | P2 | | |
| E | 75 | CD | P3 | | |
| F | 75 | CD | P3 | | |
| G | 50 | CN | ... | | |
| H | 50 | CN | ... | | |

| | | | |
|----------------------------|------------------------------|-------------------------------|---------------|
| 150: Reference Level 150 % | 50: Reference Level 50 % | CD: control ,deficient plasma | P1: patient 1 |
| 100: Reference Level 100 % | 25: Reference Level 25 % | CN: control ,normal plasma' | P2: patient 2 |
| 75: Reference Level 75 % | 12.5: Reference Level 12.5 % | | 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 interpretation results desired: |
| CONTROLS & SAMPLES | |
| 3. |  <ul style="list-style-type: none"> Pipette 100 µl of each patient's diluted plasma into the designated microwells. Pipette 100 µl of each working dilution of the Reference Plasma and the diluted Controls into the designated wells. |
| 4. |  <p style="text-align: center;">Incubate for 30 minutes at 20-26°C/68-78.8°F.</p> |
| 5. |  <p style="text-align: center;">Wash 3x with 300 µl washing buffer (diluted 1:50).</p> |

CONJUGATE

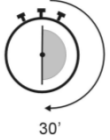
6.

CONJ



Pipette 100 µl conjugate into each well.

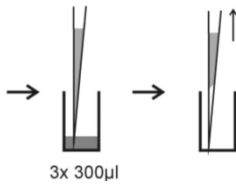
7.



Incubate for 30 minutes at 20-26°C/68-78.8°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.

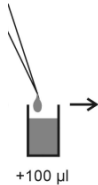


Incubate for 30 minutes at 20-26°C/68-78.8°F, protected from intense light.

STOP

11.

STOP



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

12.

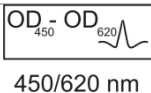


Incubate 5 minutes minimum.

13.

Agitate plate carefully for 5 sec.

14.



450/620 nm

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

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8 Quantitative Interpretation

For **quantitative interpretation** establish the reference curve by plotting the optical density (O.D.) of each dilution of the Reference Plasma (y-axis) against the corresponding value of the Reference Level in % (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the O.D. of each sample, read the corresponding patient relative value expressed in %. Multiply the patient relative value obtained from the reference curve by the assigned factor referred in the quality control leaflet to calculate the Protein C antigen level in % of normal.

Example of a standard curve

We recommend pipetting each dilution of the Reference Plasma in parallel for each run.

| Reference Level | OD 450/620 nm | Results (%) | CV % (Variation) |
|-----------------|---------------|-------------|------------------|
| 12.5 % | 0.569 | 11.95 | 1.05 |
| 25 % | 0.874 | 26.68 | 0.94 |
| 50 % | 1.163 | 48.06 | 1.04 |
| 75 % | 1.434 | 77.70 | 0.97 |
| 100 % | 1.583 | 99.61 | 1.01 |
| 150 % | 1.826 | 147.73 | 1.02 |

Example of calculation

| Patient | Replicate (OD) | Mean (OD) | Patient relative value (%) | Factor | Patient Protein C (%) |
|---------|----------------|-----------|----------------------------|--------|-----------------------|
| P 01 | 0.933/0.927 | 0.930 | 31.8 | 0.96 | 30.5 |
| P 02 | 1.790/1.810 | 1.800 | 112.3 | 0.96 | 107.8 |

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.

Expected values

The values for Protein C are given in relative percent (%) as compared to pooled normal plasma. The Protein C concentration in normal human plasma ranges usually between 70 % and 140 %. Samples with values above the range of the reference curve may be assayed again at higher dilutions for accurate 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.

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9 Technical Data

| | |
|---------------------------|---|
| Sample material: | plasma |
| Sample volume: | 20 µl plasma diluted 1:51 with 1x sample buffer |
| Total incubation time: | 90 minutes at 20-26°C/68-78.8°F |
| Calibration range: | 12.5-150 % |
| Analytical sensitivity: | 6,0% |
| 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 PROTEIN C gave an analytical sensitivity of 6.0%.

10.2 Clinical Performance

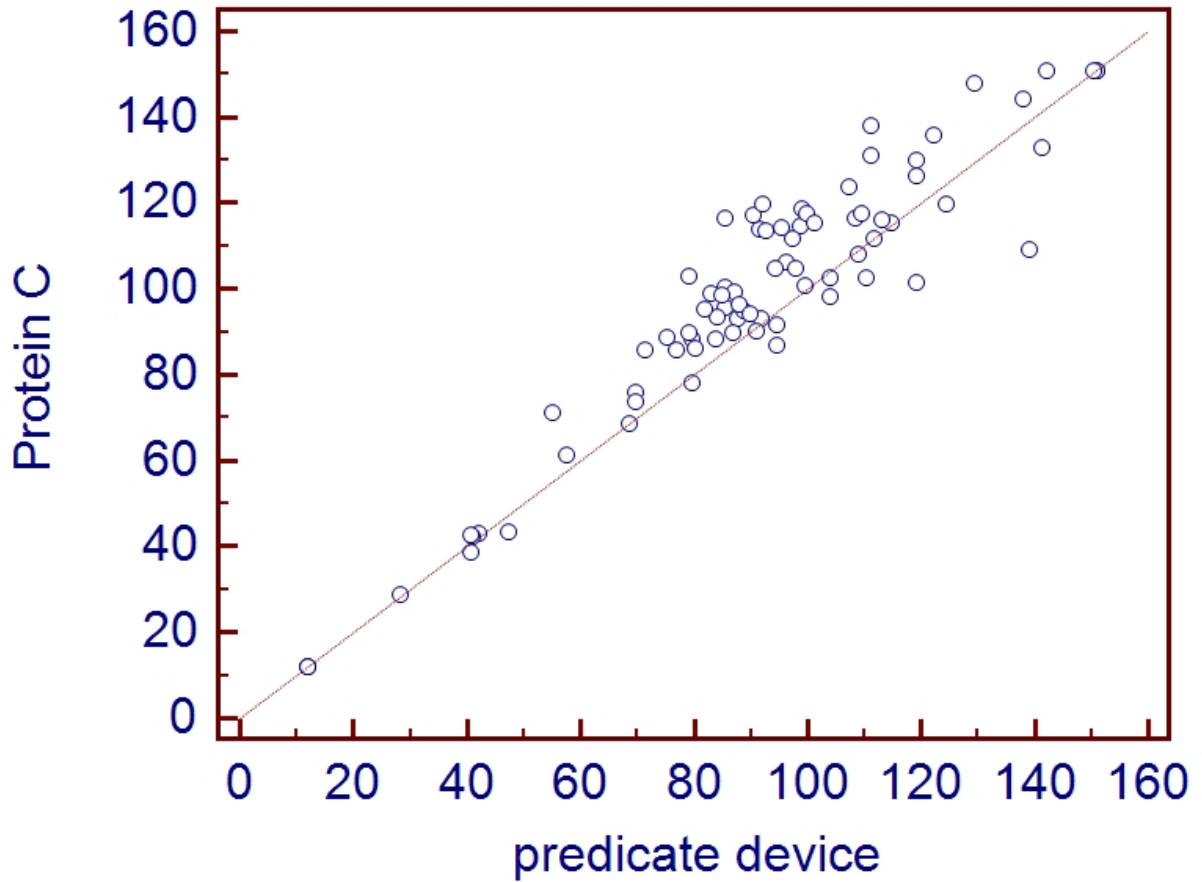
The microtitre plates are coated with a capture antibody specific for human Protein C. In accordance with laboratory diagnostic recommendations, a sample was considered deficient in the analyte when less than 70% of the normal value was measured (Labor und Diagnose; editor L. Thomas; 8th edition 2012; Frankfurt/Main; Germany).

79 plasma samples have been tested on the Protein C and a predicate device.

| Protein C | Predicate device | | | Total |
|-----------|------------------|-----|-------|-------|
| | POS | NEG | Total | |
| POS | 11 | 0 | 11 | |
| NEG | 3 | 65 | 68 | |
| Total | 14 | 65 | 79 | |

| | | |
|----------------------------|-------|----------------|
| Overall percent agreement | 96.2% | 89.4% to 98.7% |
| Positive percent agreement | 78.6% | 52.4% to 92.4% |
| Negative percent agreement | 100% | 94.4% to 100% |

The correlation between the Protein C and the predicate device resulted in a correlation coefficient of $r=0.945$.



10.3 Linearity

Chosen plasma have been tested with this kit and found to dilute linearly.

| Sample No. | Dilution Factor | Measured % | Expected % | Recovery (%) |
|------------|-----------------|------------|------------|--------------|
| 1 | 1 / 50 | 115.30 | 120 | 96.1 |
| | 1 / 100 | 60.88 | 60 | 101.5 |
| | 1 / 200 | 31.71 | 30 | 105.7 |
| | 1 / 400 | 14.41 | 15 | 96.1 |
| 2 | 1 / 50 | 41.47 | 40 | 103.7 |
| | 1 / 100 | 19.86 | 20 | 99.3 |
| | 1 / 200 | 9.48 | 10 | 94.8 |
| | 1 / 400 | 4.85 | 5 | 97.0 |

| | | |
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10.4 Precision

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

| Intra-assay | | |
|-------------|--------|--------|
| Sample No. | Mean % | CV (%) |
| 1 | 115.0 | 5.3 |
| 2 | 93.0 | 1.7 |
| 3 | 27.0 | 2.1 |

| Inter-assay | | |
|-------------|--------|--------|
| Sample No. | Mean % | CV (%) |
| 1 | 116.2 | 2.4 |
| 2 | 43.3 | 7.4 |
| 3 | 8.1 | 3.7 |

10.5 Calibration

This quantitative assay is calibrated against the WHO second international standard for Protein C. The values are given in relative percent (%) as compared to pooled normal plasma.

11 Literature




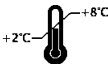

Dahlbäck B, Villoutreix BO (2005). The anticoagulant Protein C pathway. *FEBS Letters* 579: 3310-3316.

Esmon CT (2003). The Protein C Pathway. *Chest* 124: 26-32.

Miletich JP (1990). Laboratory diagnosis of Protein C deficiency. *Seminars in Thrombosis and Hemostasis* 16: 169-176.

Griffin JH, Evatt B, Wideman C, Fernandez JA (1993). Anticoagulant Protein C Pathway defective in majority of thrombophilic patients. *Blood* 82: 1989-1993.

Preissner KT (1990). Biological relevance of the Protein C system and laboratory diagnosis of Protein C and S deficiencies. *Clinical Science* 17: 351-364.

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