DRG® Akt (total), (human, mouse, rat) ELISA (EIA-3996)

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1 INTRODUCTION

AKT, also known as the protein kinase B-α (PKB-α) or RAC-PKα, was initially identified as one of the downstream targets of PI-3 Kinase (PI3-K). AKT is now known to consist of three highly conserved isoforms, which are designated in humans as AKT1, AKT2, and AKT3. Each isoform consists of an N-terminus pleckstrin homology (PH) domain, which mediates lipid-protein or protein-protein interactions, and a C-terminus kinase catalytic domain. Although each kinase is expressed differentially in a tissue-specific manner, they respond in a similar fashion to various stimuli.

AKT can be activated by a diverse array of growth factors and physiologic stimuli in a PI3-K-dependent manner. Activation of AKT kinase is a multi-step process involving both membrane translocation and phosphorylation. Activated PI3-K generates 3' phosphoinositide products, 3,4,5-triphosphates (PI-3,4,5-P_3) and PI-3,4-P_2. AKT is recruited from the cytosol to the plasma membrane through the interaction of its PH domain with these phosphoinositides. Upon membrane localization, AKT undergoes a conformational change, which makes it accessible to phosphorylation at threonine-308 and serine-473 in the kinase domain by PDK-1 and related kinases. Activated AKT then acts as a key mediator of signals for cell survival, proliferation, angiogenesis, and a number of metabolic effects of insulin. The effects of AKT activation may be mediated by modulation of expression or activity of various molecules including Bcl-2, BAD, caspase-9, endothelial nitric oxide synthase (eNOS), glycogen synthase, and transcription factors (NF-kB, Forkhead, CREB, Mdm2).

Because of its growth-promoting effects, AKT is also emerging as a central player in tumorigenesis. A number of oncogenes and tumor supressor genes act upstream of AKT to influence cancer progression. Deletion of PTEN, a tumor suppressor gene that encodes a phosphatase, correlates with increased AKT activity in several cancers. Similarly, overexpression of active Ras, Her/Neu, or AKT genes causes hyperactivation of AKT in many cancers including pancreatic, gastric, breast, ovarian and prostate adenocarcinomas.

The DRG® AKT (Total) ELISA is designed to detect and quantify the levels of AKT protein, independent of its phosphorylation state. Although performance characterization of this ELISA kit was done primarily on human cell lines, cross-reactivity of this kit with mouse and rat cells was observed. This assay is intended to detect AKT from lysates of cells and can be used to normalize the AKT content of the samples when examining quantities of phosphorylated sites on AKT using other DRG® kits (REF. EIA-3997).

This kit has been configured for research use only and is not to be used in diagnostic procedures.

READ ENTIRE PROTOCOL BEFORE USE

2 PRINCIPLE OF THE METHOD

The DRG® AKT (Total) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA).

A monoclonal antibody specific for AKT (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing AKT, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the AKT antigen binds to the immobilized (capture) antibody.

After washing, a biotin-conjugated monoclonal antibody, specific for Total AKT, is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized AKT protein captured during the first incubation.

After removal of excess detection antibody, horseradish peroxidase-labeled streptavidin (SAV-HRP) is added. This binds to the detection antibody to complete the four-member sandwich.

After a third incubation and washing to remove all the excess SAV-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color.

The intensity of this colored product is directly proportional to the concentration of AKT present in the original specimen.

3 REAGENTS PROVIDED

Note: Store all reagents at 2 - 8°C.

Reagent	96 Test Kit
AKT (Total) **Standard**: Lyophilized. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 15 mM sodium azide; 25 mL per bottle.	1 bottle
AKT Antibody-**Coated Wells**, 96 wells per plate.	1 plate
Biotin anti-AKT (Total) (**Detection Antibody**). Contains 15 mM sodium azide; 11 mL per bottle.	1 bottle
Streptavidin Horseradish Peroxidase (SAV-HRP) Concentrate, (100x). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
HRP Diluent. Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25x); 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

4 SUPPLIES - NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Cell extraction buffer (see Recommended Formulation, chapter 8.1).
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.

9. Calibrated beakers and graduated cylinders in various sizes.

5 PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bag.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 - 8°C to maintain plate integrity.
3. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
4. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
5. It is recommended that all standards, controls and samples be run in duplicate.
6. **Extracted cell lysate samples containing AKT protein should be diluted with Standard Diluent Buffer at least 1:10.** This dilution is necessary to reduce the matrix effect of the cell lysate buffer. SDS concentration should be less than 0.0 1% before adding to the plate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots.**
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. **Never** insert absorbent paper directly into the wells.
14. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between Stabilized Chromogen and metal, or color may develop.

6 SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

7 DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Buffer provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under ASSAY METHOD. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

8 PROCEDURE FOR EXTRACTION OF PROTEINS FROM CELLS

8.1 Recommended Formulation of Cell Extraction Buffer:

10 mM	Tris, pH 7.4
100 mM	NaCl
1 mM	EDTA
1 mM	EGTA
1 mM	NaF
20 mM	$Na_4P_2O_7$
2 mM	Na_3VO_4
1%	Triton X-100
10%	glycerol
0.1%	SDS
0.5%	deoxycholate
1 mM	PMSF (stock is 0.3 M in DMSO)

Protease inhibitor cocktail (e.g., Sigma Cat. # P-2714) (reconstituted according to manufacturer's guideline).
Add 250 µL per 5 mL Cell Extraction Buffer.

This buffer is stable for 2-3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the Cell Extraction Buffer should be thawed on ice. **Important:** add the protease inhibitors just before using.
The stability of protease inhibitor supplemented Cell Extraction Buffer is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

8.2 Protocol for Cell Extraction

This protocol has been applied to several cell lines of human, mouse or rat origin. Researchers should optimize the cell extraction procedures for their own applications.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at -80°C and lysed at a later date).

4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10 minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of AKT.
   For example, 4 x 10^7 Jurkat cells grown in RPMI plus 10% FBS can be extracted in 1 mL of Extraction Buffer. Under these conditions, use of 0.1-5 µL of the clarified cell extract diluted to a volume of 100 µL/well in Standard Diluent Buffer (See Assay Method) is sufficient for the detection of AKT.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
6. Aliquot the clear lysate to clean microfuge tubes. These samples are ready for assay. Lysates can be stored at -80°C. Avoid multiple freeze/thaw cycles.

9 REAGENT PREPARATION AND STORAGE

9.1 Reconstitution and Dilution of AKT Standard

Note: This AKT standard was prepared from purified, full length, recombinant human AKT expressed in S*f*21 cells.

1. Reconstitute AKT Standard with Standard Diluent Buffer. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 20 ng/mL AKT.
   Use standard within 1 hour of reconstitution.
2. Add 0.25 mL of Standard Diluent Buffer to each of 6 tubes labeled 10, 5, 2.5, 1.25, 0.6, and 0.3 ng/mL AKT.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

Standard:	Add:	Into:
20 ng/mL	Prepare as described in step 1	
10 ng/mL	0.25 mL of the 20 ng/mL std.	0.25 mL of the Diluent Buffer
5 ng/mL	0.25 mL of the 10 ng/mL std.	0.25 mL of the Diluent Buffer
2.5 ng/mL	0.25 mL of the 5 ng/mL std.	0.25 mL of the Diluent Buffer
1.25 ng/mL	0.25 mL of the 2.5 ng/mL std.	0.25 mL of theDiluent Buffer
0.6 ng/mL	0.25 mL of the 1.25 ng/mL std.	0.25 mL of the Diluent Buffer
0.3 ng/mL	0.25 mL of the 0.6 ng/mL std.	0.25 mL of the Diluent Buffer
0 ng/mL	0.25 mL of the Diluent Buffer	An empty tube

Remaining reconstituted standard should be discarded or frozen at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.

9.2 Storage and Final Dilution of Streptavidin Horseradish Peroxidase (SAV-HRP)

Please Note: The SAV-HRP 100x concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow SAV-HRP concentrate to reach room temperature. Gently mix. Pipette SAV-HRP concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of HRP Diluent for each 8-well strip used in the assay. Label as SAV-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of SAV-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused SA V-HRP concentrate to the refrigerator.

9.3 Dilution of Wash Buffer

Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved.

Dilute 1 volume of the 25x Wash Buffer Concentrate with 24 volumes of deionized water
(e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters).
Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

10 ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the Procedural Notes/Lab Quality Control section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 µL of the Standard Diluent Buffer to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards, samples or controls to the appropriate microtiter wells. Samples prepared in Cell Extraction Buffer must be diluted 1:10 or greater in Standard Diluent Buffer (for example, 10 µL sample into 90 µL buffer). While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:50 or 1:100 may be optimal. The dilution chosen should be optimized for each experimental system. Tap gently on side of plate to thoroughly mix. (See REAGENT PREPARATION AND STORAGE.)
4. Cover plate with plate cover and incubate for **2 hours at room temperature**. Alternatively, the plate may be incubated overnight at 4°C.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
6. Pipette 100 µL of Biotin anti-AKT (Total) (Detection Antibody) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.

7. Cover plate with plate cover and incubate for **1 hour at room temperature**.
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
9. Add 100 µL SAV-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in REAGENT PREPARATION AND STORAGE.)
10. Cover plate with the plate cover and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
12. Add 100 µL of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.
13. Incubate for **30 minutes at room temperature and in the dark.** ***Please Note:*** **Do not cover the plate with aluminum foil or metalized mylar.** The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
14. Add 100 µL of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of Stabilized Chromogen and Stop Solution. Read the plate within 2 hours after adding the Stop Solution.
16. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the AKT (Total) concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by dilution factor to correct for the dilution in step 3.** (Samples still producing signals higher than the highest standard (20 ng/mL) should be further diluted in Standard Diluent Buffer and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

11 TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 20 ng/mL AKT.

AKT Standard (ng/mL)	Optical Density (450 nm)
0	0.240
0.3	0.299
0.6	0.3 13
1.25	0.463
2.5	0.556
5	1.257
10	1.835
20	3.376

12 LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 20 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain.

Dilute samples >20 ng/mL with Standard Diluent Buffer; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native AKT in various matrices has not been investigated.

This kit is for research use only.

Not for human therapeutic or diagnostic use.

13 PERFORMANCE CHARACTERISTICS

13.1 SENSITIVITY

The analytical sensitivity of this assay is <0.1 ng/mL of human Total AKT. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. In Jurkat cells cultured in complete medium, this sensitivity corresponded to the AKT protein extractable from 1500 cells.

The sensitivity of this ELISA was compared to Western blotting using known quantities of AKT. The data presented in Figure 1 show that the sensitivity of the ELISA is approximately 2x greater than that of Western blotting. The bands shown in the Western blot data were developed using mouse AKT, an alkaline phosphatase conjugated anti-mouse IgG followed by chemiluminescent substrate and autoradiography.

Figure 1: Detection of AKT (Total) ELISA vs Western Blot:

Western Blot (58 kDa)								
ELISA: OD 450 nm	0.146	0.208	0.270	0.465	0.573	0.973	1.521	2.582
AKT (ng/test)	0	0.03	0.06	0.125	0.25	0.5	1	2

13.2 PRECISION

1. Intra-Assay Precision

Samples of known AKT concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	10	3.7	1.4
SD	0.7	0.3	0.09
%CV	6.9	9.9	6.3

SD = Standard Deviation

CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	10.9	3.5	1.8
SD	1.07	0.3	0.1
%CV	9.7	9.6	8.7

13.3 RECOVERY

To evaluate recovery, extract buffer was diluted 1:10 with Standard Diluent Buffer to bring the SDS concentration <0.01%. Recombinant AKT was spiked into the extract at 3 levels and the percent recovery over endogenous levels calculated. On average, 101% recovery was observed.

13.4 PARALLELISM

Natural AKT from Jurkat cells and 3T3-L1 mouse cell extracts was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the AKT standard curve. Parallelism was demonstrated and indicated that the standard accurately reflects AKT content in samples.

13.5 LINEARITY OF DILUTION

Jurkat cells were grown in tissue culture medium containing 10% fetal bovine serum and lysed with Cell Extraction Buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for AKT content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

	Extract Buffer		
Dilution	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
Neat	13.9	13.9	100
1/2	6.2	6.9	90
1/4	3.5	3.5	100
1/8	2.1	1.8	116

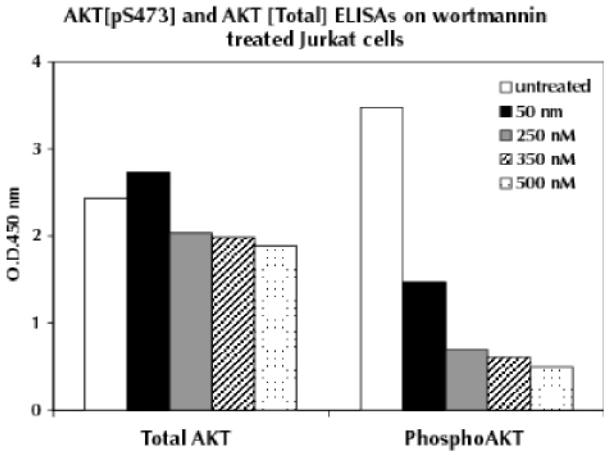
13.6 SPECIFICITY

The AKT (Total) ELISA kit is specific for measurement of total AKT protein.

The following proteins were tested in the assay at 100 ng/mL and found to have no cross-reactivity:
p38 MAPK, p42 ERK1, p42 ERK2, JNK1, human insulin receptor, rat insulin receptor, human EGFR.

In Figure 2, Jurkat cells were treated with wortmannin, a PI3-K specific inhibitor, at varying concentrations of 0 - 500 nM for 3 hours, lysed, and assayed in parallel for both AKT (Total) and AKT [pS473]. The amount of Total AKT remained comparable while the levels of phosphorylation at serine residue 473 decreased with increasing doses of wortmannin. The phosphorylated AKT was analyzed with DRG's AKT [pS473] ELISA (REF.: 3997).

Figure 2



The data indicate that the AKT (Total) ELISA detects both phosphorylated and non-phosphorylated AKT in Jurkat cells, whereas the AKT [pS473] ELISA detects phosphorylated AKT in wortmannin treated cells.

This "Total" assay is designed to allow normalization of AKT content among samples to permit interpretation of results from Phosphorylation Site-Specific AKT kits available from DRG®.

14 REFERENCES

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SYMBOLS USED WITH DRG® ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catalogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catalogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
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