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Chromogranin A (CGA)

REF CGA-ELISA-NG

www.cisbio.com/ivd-pi

Instructions for Use - ENG

IVD 96 8°C 2°C

Document reference: 05 – March 2024

1. NAME AND INTENDED USE

The product CGA-ELISA-NG is a kit for the quantitative enzymatic detection of human chromogranin A (CGA) in serum or EDTA plasma in adults.

The CGA-ELISA-NG kit is intended to be used as an aid to diagnosis in the determination of the presence and progression of GEP (Gastro-EnteroPancreatic) type neuroendocrine neoplasms (NNE), in adults.

The kit is intended for professional use and for manual use.

2. INTRODUCTION

CGA is a hydrophilic and acidic protein of 439 aa (49 kD) presents in the chromaffin granules of neuroendocrine cells. It is part of the granin family. CGA acts as a pro-hormone. Its proteolysis is a key component of its physiology. This degradation releases biologically active peptides (vasostatins, chromostatin, pancreastatin, parastatin), which possess different paracrine and autocrine functions. This proteolysis is tissue-specific and the fragmentation of the protein will be different depending on its location. It primarily takes place in the cell, inside chromaffin granules. In immunohistochemistry, the presence of CGA in tumour cells is suggestive of a neuroendocrine origin of the tumour. Circulating CGA exists in healthy subjects and the values obtained are independent of age and gender. The relevance of CGA determination in serum samples has been demonstrated for the endocrine cancers, with particularly significant elevations in gastro-entero-hepatic endocrine tumours. Studies have demonstrated that circulating CGA levels were associated with neuroendocrine differentiation and linked to the tumour mass, without, however, replacing more specific secretions.

3. PRINCIPLE

The CHROMOGRANIN A kit is an ELISA-type immunoassay. A first monoclonal antibody, immobilized on the microplate, captures the CGA proteins contained in the calibrators and samples. After washing, the captured proteins are then recognized by a second monoclonal antibody conjugated to HRP (Horse-Radish-Peroxidase). After a second incubation, the unbound reagents are eliminated by washing. Then the colorimetric reaction is started by the addition of an HRP substrate, TMB (3, 3', 5, 5' Tetramethyl benzidine). Once the reaction is stopped, the optical density (OD) of each well is read at 450 nm. The OD values measured are proportional to the CGA concentration contained in the calibrators and samples.

4. REAGENTS

Each kit contains enough reagents for 96 tests (including the generation of the calibration curve). The expiry date is indicated on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
MICROPLATE: Ready for use. Anti-CGA monoclonal mouse antibody fixed to the bottom of the well, Bovine albumin.	MICROPLATE	1 plate with 96 wells	Before opening: 2-8°C until the expiry date. After opening: unused strips may be stored for 6 weeks in the plastic bag supplied, with a desiccant, properly sealed, within the limits of the expiry date.

CONJUGATE: Ready for use Anti-CGA monoclonal mouse antibody coupled to HRP, Non-immunised mouse immunoglobulins, stabilizers and preservative.	CONJ	1 vial 22mL	Before opening: 2-8°C until the expiry date. After opening: the conjugate can be stored at 2-8°C for a period of 6 weeks , within the limits of the expiry date.
CALIBRATORS: Lyophilized. Human recombinant CGA, human serum, EDTA, preservative. 75 – 140 – 300 – 600 – 1000 ng /mL * Reconstitute with 0.25 mL of distilled water.	CAL	5 vials	Before opening: 2-8°C until the expiry date. After reconstitution: do not store for more than one hour at room temperature, divide into aliquots and freeze at - 20°C for a period of 6 weeks, within the limits of the expiry date.
CONTROLS: Lyophilized. Human recombinant CGA, human serum, EDTA, preservative. 90 – 720 ng/mL ** Reconstitute with 0.25 mL of distilled water.	CONTROL	2 vials	Before opening: 2-8°C until the expiry date. After reconstitution: do not store for more than one hour at room temperature, divide into aliquots and freeze at - 20°C for a period of 6 weeks, within the limits of the expiry date.
DIL/CAL0 : Ready for use. This reagent is used as an incubation buffer, diluent and calibrator 0. Buffer, beef serum, sodium azide, EDTA.	DIL CAL 0	1 vial 80 mL	Before opening: 2-8°C until the expiry date**.** After opening: the diluent/CAL0 can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
TWEEN 20: Concentrated washing solution Dilute 9 mL of Tween 20 in 3 L of distilled water. Shake gently.	TWEEN 20	1 vial 10 mL	Before opening: 2-8°C until the expiry date. After opening: the Tween 20 can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
SUBSTRATE: Ready for use 3, 3', 5, 5' Tetramethyl benzidine: TMB	SUBS TMB	1 vial 15 mL	Before opening: 2-8°C until the expiry date. After opening: the TMB can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
STOP SOLUTION: Ready for use 0.5 M sulphuric acid.	STOP SOLN	1 vial 22 mL	Before opening: 2-8°C until the expiry date. After opening: the stop solution can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
ADHESIVE FILM FOR MICROPLATE		3	
PLASTIC BAG		1	

(*)The values indicated above are target values only, the actual values are indicated on the vial labels.
(**) The actual acceptance limit values are indicated on the vial labels.

5. PRECAUTIONS FOR USE

5.1. Safety measures

- The raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and have been found to be negative for anti-HIV 1, anti-HIV 2 and anti-HCV antibodies and the HBs antigen. However, as it is impossible to strictly guarantee that such products are incapable of transmitting hepatitis, the HIV virus or any other viral infection, all raw materials of human origin, including the samples to be assayed, must be treated as potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which samples or kit reagents are handled. Wear disposable gloves while handling kit reagents or samples and wash hands thoroughly afterwards. Avoid splashing.
- Decontaminate and dispose of samples and all potentially contaminated materials as if they contained infectious agents. The best decontamination method is autoclaving for a minimum of one hour at 121.5°C.

- Sodium azide may react with lead or copper piping to form highly explosive metal azides.
- When disposing of waste, dilute thoroughly to prevent the formation of such products.

CAL | CONTROL | CONJ

WARNING
H317: May cause an allergic skin reaction

STOP | SOLN

Not classified as dangerous but solution with acid PH

DIL | CAL **0**

Contains sodium azide (<0.1%)

5.2. Handling precautions

- Do not use kit components beyond their expiry date.
- Do not mix reagents from different batches.
- Avoid any microbial contamination of the reagents and water. Comply with the incubation times.

6. SAMPLE COLLECTION AND PREPARATION

6.1 Pre-analytical

The assay is performed directly on serum or EDTA plasma. For an assay performed within 4 hours, the samples must be stored at room temperature (18-25°C). For an assay performed within 48 hours, the samples must be stored at 2-8°C following specimen collection. For an assay beyond 48 hours, samples should be divided into aliquots which must be stored frozen (-20°C) up to 10 months.

Dilution: If high CGA levels are suspected, dilution should be performed using the diluent buffer supplied with the kit. It is recommended that dilutions be performed in disposable plastic tubes.

6.2 Pre-dilution of samples, controls and calibrators (1/51)

- **All the samples, the controls and the calibrators must be pre-diluted 51 times** in the diluent DIL | CAL 0 provided in the kit before being tested. Gently mix the mixture using a Vortex mixer.

7. ASSAY PROCEDURE

7.1 Equipment required

- Precision micropipettes or similar equipment with disposable tips for distribution of 20, 50, 100, 200 and 1000 µL. Calibration of these must be regularly checked.
- Distilled water.
- Disposable plastic tubes.
- Vortex mixer.
- Microplate washer (optional).
- Microplate shaker.
- Microplate reader, capable of measuring absorbance at 450 nm. As an option, the reader may be equipped with a filter capable of reading the absorbance at a wavelength between 610 nm and 650 nm (620 nm recommended). This second reading allows to correct the microplate's imperfections.

7.2 Protocol

- All the reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. The reagents are pipetted and dispensed into wells at room temperature (18-25°C).
- Each calibrator, control or sample must be tested in duplicate.
- Reconstitute the vials of calibrators and controls. Carefully check that all the freeze-dried product is dissolved, and use within an hour following reconstitution.

7.2.1 Preparation of the Wash solution TWEEN 20

- To obtain reliable and reproducible results, it is recommended that the washing steps be performed as indicated; the residual washing solution volume must be as low as possible. The use of a microplate washer is recommended.
  - To prepare the washing solution, dilute 9mL of Tween 20 TWEEN 20 in 3L of distilled water. Mix slowly.

7.2.2 Instructions - Follow the order for addition of reagents

See last page for laboratory protocol card. It is necessary to fully read the package insert in details before using the laboratory protocol card.

1. Prepare and identify a sufficient number of test tubes to perform a pre-dilution of samples, calibrators and controls
2. Determine the number of microtiter well strips required for the assay. Remove unused strips from the frame holder and store them at 2-8°C in the adhesive bag, properly sealed with a desiccant.
3. **Pre-dilute the calibrators, samples and controls in plastic tubes to 1:51**

   a. Dispense 1mL of diluent DIL CAL 0 into the plastic tubes
   b. Add 20µL of <u>each</u> calibrator, control or sample and gently mix with a vortex-type mixer
4. Dispense 200 µL of pre-diluted calibrators CAL, samples or controls CONTROL to 1/51 into the DIL/CAL0 DIL CAL 0 into each wells.
5. Cover with the adhesive film, agitate for **1h at 700 rpm** at room temperature **(18-25°C).**
6. Wash the wells as follows:

   a. Remove the content of the wells
   b. Dispense 300 µL of wash solution TWEEN 20 prepared as described in chapter 7.2.1
   c. Repeat steps a. and b. 2 times more for a total of 3 washing cycles.
   d. Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.
7. Dispense **200 µL** of HRP conjugate CONJ in all the wells.
8. Cover with the adhesive film and **incubate for 2h +/- 5'** at room temperature (18-25°C) under **agitation at 700 rpm.**
9. Wash the wells as above <u>then:</u>
10. Dispense 100 µL of TMB SUBS TMB in all wells. Cover with the adhesive film. Incubation in darkness is not necessary.
11. Allow the colorimetric reaction to develop **for exactly 10 min** at room temperature (18-25°C), **under agitation (700 rpm**).
12. Stop the reaction by adding 50 µL of stop solution STOP SOLN to all wells.

➢ Read off the absorbance at 450 nm. Perform a second reading (optional) of the absorbance at a wavelength between 610 nm and 650 nm.

8. QUALITY CONTROL

Good Laboratory Practices (GLP) require that quality control samples be used in each series of assays to verify the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

1. Optional OD correction*: subtract readings at 620 nm from the readings at 450 nm.

2. For each duplicate, calculate the mean absorbance (OD) of calibrators, controls and samples.

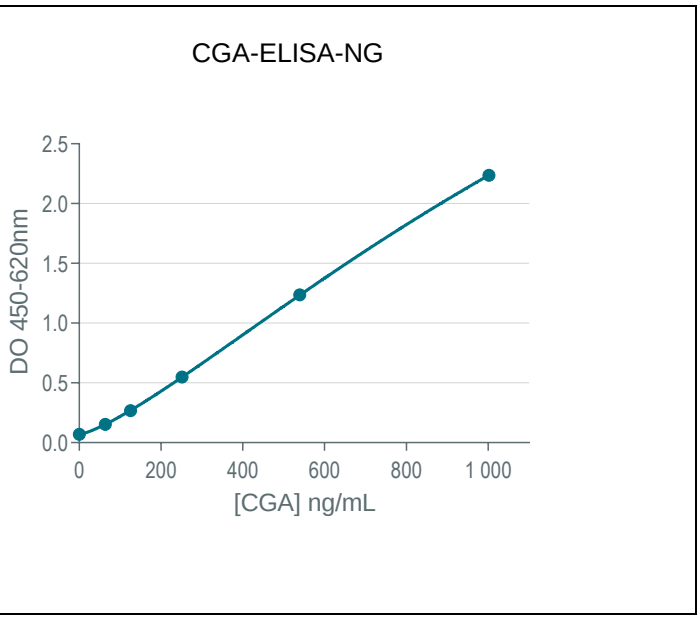
3. Build a calibration curve by plotting the (corrected*) mean OD values at 450 nm of calibrators (y-axis) against their concentration (x-axis) indicated on the vial.

4. The four parameters logistic 4PL with $1/y^2$ weight mathematical fitting model is recommended for the calibration curves. Other data reduction functions may give slightly different results.

Read the concentration of the samples from the curve. The 1:51 predilution ratio is already calculated in calibrator concentrations.

*Example of assay data**: for illustration only** and must under no circumstances be substituted for results obtained in the laboratory.*

Calibrators	Mean (OD)*	Concentrations (ng/mL)
CAL 0	0.069	0
CAL 1	0.154	64
CAL 2	0.268	125
CAL 3	0.548	251
CAL 4	1.236	539
CAL 5	2.236	1002
Control 1	0.190	83.6
Control 2	1.316	573



10. LIMITATION OF THE PROCEDURE

- Samples presenting cloudiness, haemolysis, hyperlipemia or containing fibrin may give inaccurate results.
- Do not extrapolate sample values beyond the last standard. Dilute the high concentrations samples and retest.
- Do not use the CGA-ELISA-NG kit for the determination of circulating CGA in patients with ongoing treatments based on proton-pump inhibitory drugs or in patients with decreased renal function or with atrophic gastritis. These patients have physiologically elevated levels of circulating CGA unlinked to the presence of a neuroendocrine tumor.
- Do not interpret results in patients on somatostatin analogue therapy, these patients may present with falsely low results.

11. PERFORMANCE CHARACTERISTICS

11.1 Imprecision

Samples	n	Concentration Mean (ng/mL)	Within-series (CV%)
1	34	81.6	6.43
2	36	122	4.68
3	31	182	4.13
4	35	407	3.19
5	36	445	3.98
6	35	632	4.73

Samples	n	Concentration Mean (ng/mL)	Between-series (CV%)
1	28	51.3	11.5
2	28	187	6.4
3	28	442	6.8
4	20	697	7.0

11.2 Recovery test

Known quantities of CGA were added to human sera. The recovery percentages in the samples ranged between 90 and 110%.

11.3 Dilution test

Samples with high concentrations were diluted. The recovery percentages obtained were between 80% and 120%.

11.4 Specificity

No interference was observed when serum samples were tested with any of the following substances:

- Glucagon (up to 3000ng/mL)
- Gastrin (up to 3000ng/mL)
- Chromogranin B (up to 3000ng/mL)
- NSE (up to 3000ng/mL)
- Pancreatic polypeptide (up to 3000ng/mL)

11.5 Measurement range

The samples must be measured in the range between the limit of quantitation and the highest concentration of the calibration range, i.e. between 30.6 and 1000 ng/mL.

11.6 Limit of detection

The limit of detection (LOD or analytical sensitivity) of the CGA-ELISA-NG kit is defined as being the lowest detectable concentration that differs from zero with a probability of 95% calculated by adding 2 standard deviations to the mean value of 30 replicate analysis of the zero calibrator (CAL0). It was measured at 16.9 ng/mL.

The functional sensitivity is defined as being the concentration measured by the imprecision profile at a CV equal to 20%. It is estimated to be 30.6 ng/mL.

11.7. Hook effect

There is no hook effect up to 1.000.000 ng/mL.

11.8. Interference

- When the assay protocol provided in the instructions for use is followed, no interference with biotin for concentration ranging from 0 to 600 ng/mL is measured.
- NOTE: Results showed that a concentration of biotin at 1200ng/mL caused a slight interference (-14% maximum bias) with the CGA-ELISA-NG kit.
- No interference with **bilirubin and hemoglobin** measured up to respective concentrations of 0.15mg/mL, 2mg/mL was observed.
- No interference was observed when serum samples were supplemented with triglycerides from hyperlipidemic human sample and tested (743.4mg/dL total TG).

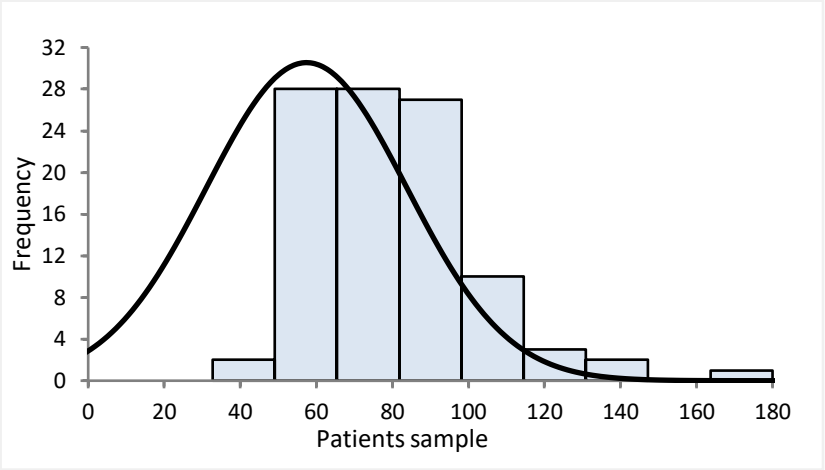
CAUTION: The immunoassay is protected against potential interferences with **heterophilic antibodies** such as HAMA and rheumatoid factors (RF) by addition of a protection (non-specific mouse immunoglobulins). Nevertheless, we cannot assure that there will never be any false positive or negative result due to the presence of heterophilic antibodies and rheumatoid factors in patient samples.

12. EXPECTED NORMAL VALUES

It is recommended that each laboratory determines its own normal values range depending on the type of sample commonly used. Chromogranin A is a calcium-binding protein and its circulating levels are affected by the Ca++ concentration. The normal human values found may differ depending on whether sera collected on dry blood collection tubes or EDTA plasmas are assayed.

The values presented below are given as indication only and were obtained on serum samples with a population of 101 presumed healthy subjects.

For the normal values distribution presented below, the 95th percentile is located at 101ng/mL.



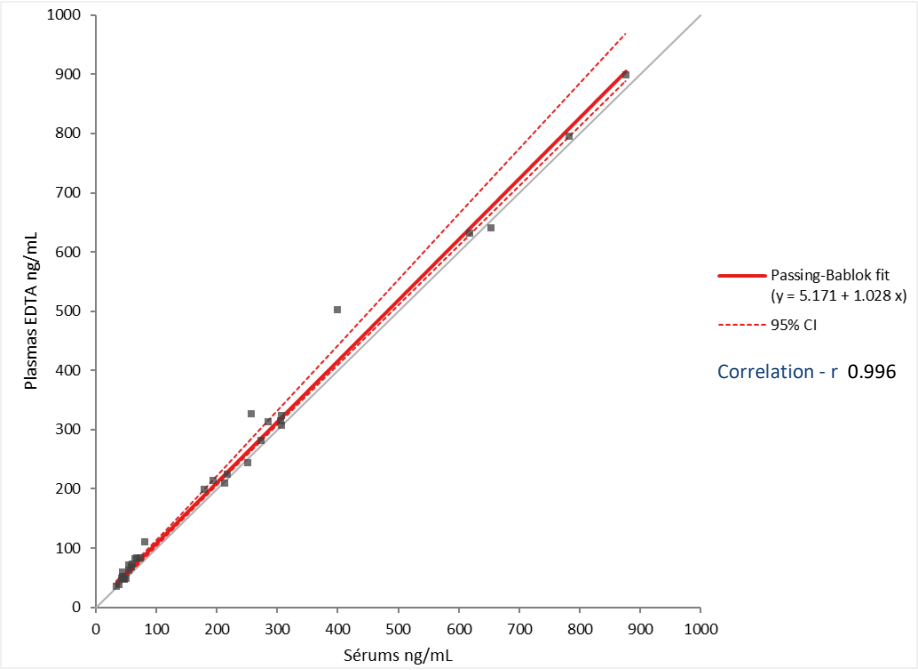
Normal values on EDTA plasma samples:

The serum/plasma correlation presented here below must be used to determine the plasma concentration of CGA.

The equation of the correlation is as follows:

[Plasma sample] = 1.028 x [Serum sample] + 5.171

To extrapolate the values on EDTA plasma, this equation should be applied to the normal values found on serum samples,



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CGA-ELISA-NG

IVD

LABORATORY PROTOCOL CARD

Do not use this card without having read the whole package insert.

Pre-dilute the calibrators, samples and controls in plastic tubes to 1:51. **1:51 pre-dilution**

1. DISPENSE 1mL of diluent into the plastic tubes DIL CAL 0

2. ADD 20µL of each calibrator, control or sample and gently mix with a vortex-type mixer CAL CONTROL

3. DISPENSE SAMPLES INTO THE MICROPLATE CAL CONTROL

⇓ Dispense 200 µL of **pre-diluted calibrators, samples or controls to 1/51** into the DIL/CAL0 into each wells (Distribution in duplicate).

+ 200 µL

4. AGITATION

⇓ Cover with the adhesive film, **agitate for 1H at 700 rpm** at room temperature **(18-25°C).**

700 rpm **1h**

5. WASH THE WELLS (see § 7.2.1) TWEEN 20

Prepare the wash solution by dilution of 9mL of Tween 20 in 3L of distilled water.
Remove the content of the wells.
Dispense 300µL of wash solution prepared into each well
Repeat steps 2 times more for a total of 3 washing cycles
Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.

3 x 300 µL

6. DISPENSE THE CONJUGATE CONJ

Dispense 200µL of HRP conjugate in all the wells

+ 200 µL

7. INCUBATE

Cover with adhesive film and incubate for **2h +/-5'** at room temperature (18-25°C) **under agitation at 700 rpm.**

700 rpm **2h +/-5'**

8. WASH THE WELLS (see § 7.2.1) TWEEN 20

Prepare the wash solution by dilution of 9mL of Tween 20 in 3L of distilled water.
Remove the content of the wells.
Dispense 300µL of wash solution prepared into each well.
Repeat steps 2 times more for a total of 3 washing cycles.
Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.

3 x 300 µL

9. DISPENSE THE SUBSTRATE SUBS TMB

⇓ Dispense 100µL of TMB in all wells. Cover with the adhesive film. Incubation in darkness is not necessary.
Allow the colorimetric reaction to develop **for exactly 10 min** at room temperature (18-25°C) **under agitation at 700 rpm**

+ 100 µL

700 rpm

10 min

10. DISPENSE THE STOP SOLUTION STOP SOLN

⇓ Stop the reaction by adding 50µL of stop solution to all wells.

+ 50 µL

11. READ

Read off the absorbance at **450 nm.** Perform a second reading (optional) of the absorbance at a wavelength of 620nm (between 610 and 650 nm).
Use a **balanced 4-parameters logistic** fit for data interpolation.

450 nm

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MISE A JOUR / UPDATING

CE

CGA-ELISA-NG
Cisbio Bioassays – Mars 2024 - Modèle 05

FRA

Modifications par rapport à la version précédente :
Nouveau logo Revvity; mise à jour de l'adresse email de l'assistance "in vitro"

ENG

Changes from the previous version:
New Revvity logo; update of the IVA email address.

DEU

Änderungen gegenüber der Vorgängerversion:
Neues Revvity-Logo; aktualisierte E-Mail-Adresse für "In-vitro"-Support

ITA

Modifiche rispetto alla versione precedente:
Nuovo logo Revvity; aggiornamento dell'indirizzo e-mail del supporto in vitro

SPA

Cambios desde la versión anterior:
Nuevo logotipo de Revvity; dirección de correo electrónico de asistencia in vitro actualizada

HUN

Változások az előző verzióhoz képest:
Új Revvity logó; frissített in vitro támogatási e-mail cím

CES

Změny od předchozí verze:
Nové logo Revvity; aktualizovaná e-mailová adresa podpory in vitro

POR

Alterações em relação à versão anterior:
Novo logótipo Revvity; endereço de correio eletrónico de apoio in vitro atualizado

	FRA	ENG	DEU	ITA	SPA	HUN	CES	POR
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Significado de los simbolos**	**Jelmagyarázat**	**Vysvětlení symbolů**	**Significadodos simbolo**
	Limite de température	Temperature limitation	Temperaturbegrenzung	Limiti di temperatura	Limitación de temperatura	Tárolási hőmérséklethatár	Mezní teplota skladování	Limite da temperatura de armazenagem
	Code du lot	Batch code	Chargencode	codice lotto	Código de lote	Gyártási szám	Č. šarže	Lote
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Fecha de caducidad	Felhasználható az alábbi dátumig :	Použitelné do	Utilizado por
	Consulter la notice d'utilisation	Consult instructions for use	Das Handbuch zu Rate ziehen	Consultare le istruzioni per l'uso	Consúltense las instrucciones de uso	Olvassa el a használati utasítást	Přečtěte si návod k použití	Consulte o manual de operações
	Dispositif médical de diagnostic in vitro	In vitro medical device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Producto sanitario para diagnóstico in vitro	In vitro diagnosztika	Diagnostika in vitro	Dispositivo de diagnostico In Vitro
	Fabricant	Manufacturer	Hersteller	Fabbricante	Fabricante	Gyártó	Vyrobil	Fabricado por
	Référence du catalogue	Catalogue number	Katalog Nr.	N. catalogo	Número de catálogo	Referenciakészítmény	Reference	Número do catalogo
	Suffisant pour	Sufficient for	Ausreichend für	Sufficiente per	Válido para	A kémcsövek száma	Počet zkumavek	Suficiente para
	Conserver à l'abri de la lumière du soleil	Keep away from sunlight	Vor Sonnenlicht schützen	Conservare al riparo dalla luce solare	Manténgase fuera de la luz del sol	Napfénytől védve tárolandó	Chraňte před slunečním světlem	Manter afastado da luz solar
	Risques biologiques	Biological Risks	Biogefährdung	Rischio biologico	Riesgos biológicos	Biológiai veszély	Biologické riziko	Riscos Biológicos
CONJ	Conjugué	Conjugate	Komplex	Coniugato	Conjugado	Kétfázisű elegy	Konjugát	Conjugado
CAL	Calibrateur	Calibrator	Kalibrator	Calibratore	Calibrador	Kalibrátor	Kalibrátor	Calibrador
CONTROL	Contrôle	Control	Kontrolle	Controllo	Control	Kontroll	Kontrola	Controle
TWEEN 20	Solution concentrée	Concentrated solution	Konzentrierte Lösung	Soluzione concentrata	Solución concentrada	Koncentrált oldat	Koncentrovaný roztok	Solução concentrada
MICROPLATE	Microplaque	Microplate	Mikrotiterplatte	Micropiastra	Microplaca	mikrolemez	Mikrotitrační destička	Microplaca
DIL CAL	Diluant	Diluent	Verdünnungs-mittel	Diluente	Diluyente	Hígítószer	ředidlo	Diluente
SUBS TMB	Substrat	Substrate	Substrat	Sustrato	Substrato	Szubsztrátum	Substrát	Substrato
STOP SOLN	Solution d'arrêt	Stop solution	Stopplösung	Soluzione d'arresto	Solución de parada	Semlegesitó oldat	Zastavovacó roztok	Solução de paragem