Novembre 2017 – Modèle 22

Cisbio Bioassays
Parc Marcel Boiteux - BP 84175 – 30200 Codolet / France - Tél. 33 (0)4.66.79.67.00

RIA-gnost® CA-50

REF *OCFM07-CA50*

Σ 100

IVD

CE

2°C – 8°C

Trousse pour la détermination radioimmunologique de l'antigène CA-50 dans le sérum ou le plasma

Pour diagnostic In Vitro

La trousse contient :

Tubes revêtus	2 x 50
Traceur ≤ **300 kBq**	1 x 22 ml
Calibrateur 0	1 x 0,5 ml
Calibrateurs 1 - 6	6 x 0,5 ml
Sérums de contrôle	2 x 0,5 ml
Tampon d'incubation	1 x 25 ml
Réactif de lavage	1 x 5 comprimés
Sachet plastique	1
Notice d'utilisation	1

Attention : Certains réactifs contiennent de l'azoture de sodium

Kit for the radioimmunological determination of CA-50 antigen in serum or plasma

For In Vitro diagnostic use

Kit content :

Coated tubes	2 x 50
Tracer ≤ **300 kBq**	1 x 22 mL
Calibrator 0	1 x 0.5 mL
Calibrators 1 – 6	6 x 0.5 mL
Control serum	2 x 0.5 mL
Assay buffer	1 x 25 mL
Wash reagent	1 x 5 tablets
Plastic bag	1
Instruction for use	1

Warning : Some reagents contain sodium azide

Kit zur radioimmunologischen Bestimmung von CA-50-Antigen in Serum oder Plasma

Zur In Vitro diagnostik

Inhalt des Kits :

Teströhrchen beschichtet	2 x 50
Tracer ≤ **300 kBq**	1 x 22 ml
0 – Kalibrator	1 x 0,5 ml
Kalibratoren 1 - 6	6 x 0,5 ml
Kontrollserum	2 x 0,5 ml
Assaypuffer	1 x 25 ml
Waschreagenz	1 x 5 Tabletten
Plastikbeutel	1
Gebrauchsinformation	1

Achtung : Einige Reagenzien enthalten Natriumazid

Kit per il dosaggio radioimmunologico dell'antigene CA-50 nel siero o nel plasma

Per uso diagnostico In Vitro

Contenuto del kit :

Provette coattate	2 x 50
Tracciante ≤ **300 kBq**	1 x 22 ml
Calibratore 0	1 x 0,5 ml
Calibratori 1 – 6	6 x 0,5 ml
Siero di controllo	2 x 0,5 ml
Tampone di incubazione	1 x 25 ml
Reagente di lavaggio	1 x 5 compresse
Sacchetto di plastica	1
Istruzioni per l'uso	1

Attenzione : Alcuni reagenti contengono sodio azide

Equipo para la determinación radioinmunológica del antígeno CA-50 en suero o en plasma

Para uso de diagnóstico In Vitro

Contenido del equipo :

Tubos recubiertos	2 x 50
Trazador ≤ **300 kBq**	1 x 22 ml
Calibrador 0	1 x 0,5 ml
Calibradores 1 – 6	6 x 0,5 ml
Suero control	2 x 0,5 ml
Tampón de incubación	1 x 25 ml
Reactivo de lavado	1 x 5 comprimidos
Bolsa de plástico	1
Instrucciones de uso	1

Precauciones : Algunos reactivos contienen azida sódica

Τυποποιημένη συσκευασία για το ραδιοανοσολογικό προσδιορισμό του αντιγόνου CA-50 στον ορό ή στο πλάσμα

Για διαγνωστική χρήση in vitro

Περιεχόμενα της τυποποιημένης συσκευασίας

Επικαλυμμένοι σωλήνες	2 x 50
Ιχνηθέτης ≤ **300 kBq**	1 x 22 ml
Πρότυπο 0	1 x 0,5 ml
Πρότυπα 1 – 6	6 x 0,5 ml
Ορός μάρτυς	2 x 0,5 ml
Ρυθμιστικό διάλυμα	1 x 25 ml
Αντιδραστήριο έκπλυσης	1 x 5 δισκία
Πλαστική θήκη	1
Οδηγίες χρήσεως	1

Προσοχή : Ορισμένα αντιδραστήρια περιέχουν αζίδιο νατρίου

	FRA	ENG	DEU	ITA	SPA	ELL	POR	HUN	CES	RUS
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Significado de los simbolos**	**Επεξήγηση των συμβόλων που**	**Significadodos simbolos**	**Jelmagyarázat**	**Vysvětlení symbolů**	**Объяснение символов**
	Conforme aux normes européennes	European conformity	CE-Konformitäts-kennzeichnung	Conformita europea	Conformidad europea	European conformity	Conformidad com as normas europeias	Megfelel az európai szabványoknak	Evropská shody	Европейский соответствия
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Limites de temperatura de almacenamiento	Περιορισμός θερμοκρασίας φύλαξης	Limite da temperatura de armazenagem	Tárolási hőmérséklethatár	Mezní teplota skladování	Ограничение температуры хранения
	N° de lot	Batch code	Chargencode	codice lotto	Código de lote	Κωδικός παρτίδας	Lote	Gyártási szám	Č. šarže	код партии
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Consumir antes de	Ημερομ. λήξης	Utilizado por	Felhasználható az alábbi dátumig :	Použitelné do	Используйте по
	Consulter la notice d'utilisation	Consult operating instructions	Das Handbuch zu Rate ziehen	consultare le istruzioni per l'USO	Consultar las instrucciones de manejo o funcionamiento	Ανατρέξτε στις οδηγίες λειτουργίας	Consulte o manual de operações	Olvassa el a használati utasítást	Přečtěte si návod k použití	Обратитесь к инструкции по эксплуатации
	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Dispositivo de diagnóstico In Vitro	διαγνωστική συσκευή In Vitro	Dispositivo de diagnostico In Vitro	In vitro diagnosztika	Diagnostika in vitro	В устройстве Витро диагностики
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Fabricado por	Κατασκευάζεται από την	Fabricado por	Gyártja:	Vyrobil	Изготовитель
	Référence	Catalogue number	Katalog Nr.	N. catalogo	Número de catálogo	Αριθμός καταλόγου	Número do catalogo	Referenciakészítmény	Reference	номер по каталогу
	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Número de determinaciones	Αριθμός προσδιορισμών	Número de determinações	A kémcsövek száma	Počet zkumavek	Количество определений
	Tubes revêtus	Coated tubes	beschichtete Röhrchen	Provette coattate	Tubos recubiertos	Επιστρωμένα σωληνάρια	Tubos adsorvidos	Bevont kémcsövek	Zkumavky	Покрытые трубы
	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Tracciante radioattivo	Trazador radiactivo	Ραδιενεργός ιχνηθέτης	Marcador radioativo	Nyomjelző izotóp	Tracer	радиоактивного индикатора
	Calibrateur	Calibrator	Kalibrator	Calibratore	Calibrador	Βαθμονομητής	Calibrador	Kalibrátor	Kalibrátor	калибратор
	Contrôle	Control	Kontrolle	Controllo	Control	Ορός ελέγχου	Controle	Kontroll	Kontrola	контроль
	Solution de lavage	Wash solution	Waschlotion	Soluzione di lavaggio	Solución de lavado	Διάλυμα πλύσης	Solução de lavagem	mosóoldat	promývací reagencie	Промывочный раствор
	Tampon d'incubation	Incubation buffer	Inkubationspuffer	Tampone di incubazione	Tampón de incubación	ρυθμιστικό διάλυμα επώασης	tampão de incubação	Inkubációs puffer	pufr	Инкубационный буфер

MISE A JOUR / UPDATING

CE

OCFM07-CA50
Cisbio Bioassays – Novembre 2017 - Modèle 22

FRA **Modifications par rapport à la version précédente :**
Nouveau logo / 7.2 ajout de l'information sur les essais en double

ENG **Changes from the previous version:**
New logo / 7.2 information on assays in duplicate added

DEU **Änderungen gegenüber der Vorgängerversion:**
Neues Logo / 7.2 Informationen zu Tests in Doppelbestimmungen hinzugefügt

ITA **Modifiche rispetto alla versione precedente:**
Nuovo logo / 7.2 Aggiunta informazione sui dosaggi in doppio

SPA **Cambios desde la versión anterior:**
Nuevo logotipo / 7.2 Se ha añadido información sobre los ensayos por duplicado

ELL **Αλλαγές από την προηγούμενη έκδοση:**
νέο λογότυπο / 7.2 Προσθήκη πληροφοριών σχετικά με τις δοκιμασίες εις διπλούν

POR **Alterações em relação à versão anterior:**
Novo logótipo / 7.2 Recomenda-se executar o ensaio em duplicado

HUN **Változások az előző verzióhoz képest:**
új logó / 7.2 a kétszeres assay-kre vonatkozó információ hozzáadva

CES **Změny od předchozí verze:**
Nové logo / 7.2 Je doporučeno, aby měření probíhalo v duplikátech

RUS **Изменения по сравнению с предыдущей версией:**
новый логотип / 7.2 анализы в двух экземплярах для калибраторов, контрольных образцов и образцов

1. NAME AND INTENDED USE

RIA-gnost® CA-50 (**OCFM07-CA50**) is a kit for the radio-immunological assay of CA-50 antigen in serum or plasma.
The kit is intended for professional use.

2. INTRODUCTION

Tumor cells express substances in the cell membrane which are not usually produced in healthy cell membranes. The determination of these tumor-associated structures is a valuable tool in the diagnosis of malignant disorders. Using the hybridoma technique of Köhler and Milstein, specific immunological reagents (monoclonal antibodies, MAB) can be obtained which recognize tumor-associated antigens. A monoclonal antibody of this type, C-50 MAB, was obtained after immunization using a colorectal adenocarcinoma cell line, Colo 205. The C-50 MAB recognizes two different carbohydrate chains, the sialylated Lewis-a and the hitherto unknown sialylated lactotetraose. Structures containing CA-50 are mainly found in gastrointestinal cancers (e.g. pancreatic, stomach, hepatic and colorectal cancers) but also sometimes in other malignant growths (endometrial cancers). The CA-50 antigens occur in the cell membrane in a lipid-bound form (as ganglioside) and in a form bound to a high molecular weight protein (as glycoprotein). The CA-50 antigens are released by the tumors into the blood stream where they can be specifically detected by means of immunological techniques based on C-50 MAB.

2.1. Clinical significance of CA-50 assay

CA-50 is normally detectable only in low concentrations in the serum of healthy men and women. A slight elevation in CA-50 concentrations can sometimes be observed in patients with benign disorders.
Pathologically raised serum CA-50 levels are encountered in the presence of CA-50-producing tumors, e.g. tumors of the pancreas, gastrointestinal tract, endometrium and bladder.

2.2. Malignant disorders

If a malignant disorder is suspected and elevated CA-50 levels are observed, additional tests are recommended. The main indication for determination of CA-50 is the follow-up of tumor patients, i.e. the monitoring of treatment efficacy and the monitoring of the course of the disease over time and determination of its prognosis.

2.3. Benign disorders

Benign disorders can lead to CA-50 values above the normal range; this frequently occurs, for example, in cases of acute and chronic pancreatitis, ulcerative colitis, Crohn's disease, cirrhosis of the liver and hepatitis.

3. PRINCIPLE

The RIA-gnost® CA-50 kit enables in vitro assay of the CA-50 antigen in human serum (or plasma) using the 2-step "sandwich" test principle. A complex of anti-CA-50 antibodies (monoclonal, mouse) bound to the tube wall, CA-50 in the sample and ^{125}I-labeled anti-CA-50 antibodies (monoclonal, mouse) is formed. After the reaction, the free tracer fraction is removed by decanting (or aspiration) and washing.
The amount of tracer specifically bound to the coated test tubes is measured with a gamma scintillation counter.
Evaluation of results provided by unknown samples is performed by reading off from a standard curve constructed under identical conditions.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
COATED TUBES : ready for use. anti-CA-50 mouse monoclonal antibody.	CT	2 packs of 50 tubes	2-8°C until the expiry date. Any unused coated tubes removed from their pack must be stored in the plastic bag supplied with the kit.
^{125}I-ANTI-CA-50 : ready for use anti-CA-50 ^{125}I mouse monoclonal antibody, bovine immunoglobulins, non-specific mouse immunoglobulins, bovine albumin, sodium azide, buffer, red dye. ≤ 300 kBq (≤ 8.10 µCi)	TRACER	1 22-mL vial	2-8°C until the expiry date.
CALIBRATOR 0 : ready for use buffer, sodium azide.	CAL	1 0.5-mL vial	2-8°C until the expiry date.
CALIBRATORS : ready for use. Human serum, human antigen, buffer, sodium azide. 5 – 10 – 20 – 40 – 80 – 180 U/mL (*)	CAL	6 0.5-mL vials	2-8°C until the expiry date.
CONTROLS : ready for use. Human serum, human antigen, sodium azide. (**)	CONTROL	2 0.5-mL vials	2-8°C until the expiry date.
INCUBATION BUFFER : ready for use. Buffer, bovine albumin, bovine immunoglobulins, sodium azide, blue dye.	BUF	1 25-mL vial	2-8°C until the expiry date.

WASHING REAGENT : tablets Dissolve the 5 tablets in 500 mL of distilled water. Shake.	**BUF-WASH**	1 blister of 5 tablets	2-8°C until the expiry date.
PLASTIC BAG		1	
PACKAGE INSERT		1	

(*) The values indicated above are target values: the true values are indicated on the labels of the vials. Calibration was performed relative to a reference preparation (arbitrary system).
(**) The true values of the acceptance limit are given on the vial label.

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 still 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.
Some reagents contain sodium azide as a preservative. Avoid any absorption of the reagents, as well as any contact with the skin or mucous membranes. Sodium azide may react with lead or copper piping to form highly explosive metal azides. Dilute well when disposing of waste.

5.2. Basic radiation protection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use and exchange of radioactive products are subject to the laws in force in the user's country. Enforcement of the basic radiation protection rules will ensure adequate security.
A summary of these is given below:
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions. Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves. Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes. Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures. All radioactive waste disposal must be carried out according to the regulations in force

5.3. 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 or of the water used for washing. Fully respect the incubation times and the washing instructions indicated

6. SAMPLE COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma. If the test is to be carried out within 3 days after the sample is taken, the serum or plasma samples can be stored at 2-8°C.
If not, they should be divided into aliquots which must be stored frozen (-20°C).
Avoid re-freezing a sample after defrosting. After defrosting or removal from the refrigerator, shake the samples well.
Dilutions: If high CA-50 levels are suspected, dilutions should be prepared with incubation buffer.

7. ASSAY PROCEDURE

7.1. Equipment required

200 µL and 50 µL micropipettes with interchangeable tips, graduated test tubes, horizontal agitation system, distributor: 1 mL, gamma counter set for the measurement of iodine 125.

7.2. Assay protocol

It is recommended to perform the assays in duplicate for calibrators, controls and samples.
Bring the components of the kit stored at between 2 and 8°C to room temperature (between 18 and 25°C) before use. To prepare the washing buffer, dissolve the 5 buffer tablets in 500 mL distilled water.
Store all unused reagents at a temperature of between 2 and 8°C. Any unused coated tubes removed from their pack must be stored in the plastic bag supplied with the kit.

1. Number a sufficient quantity of antibody-coated tubes, as indicated in the table (calibrators, control serum, patient samples).
2. Distribute 50 µL of calibrator, controls, or sample to be assayed at the bottom of the tubes prepared for this purpose. Use a new pipette tip for each sample.
3. Distribute 200 µL of buffer in each tube.
4. Shake the tubes on a horizontal agitation system (300 rpm) for 2 hours at a temperature of between 18 and 25°C.
5. Distribute 1 mL of washing buffer in each tube, decant (aspirate) and wash with 1 mL washing buffer.
6. Distribute 200 µL ^{125}I-labeled anti-CA-50 in each tube.

7. Shake the tubes on a horizontal agitation system for 2 hours at a temperature of between 18 and 25°C.
8. Distribute 1 mL of washing buffer in each tube, decant (aspirate), repeat the operation once.
9. Measure the radioactivity of the tubes for 1 minute using a gamma counter.

Comment
To perform a larger series of assays, the reagents from several kits from the same batch should be pooled.

8. QUALITY CONTROL

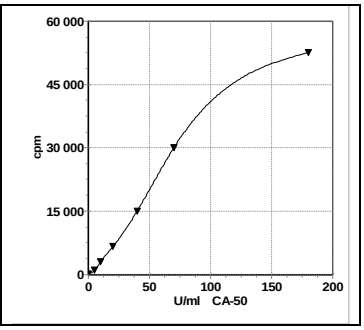
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All samples should be treated in the same way as the samples to be assayed and analysis of the results using appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, subtract the background noise and calculate the mean of the counts. Draw up the standard curve by plotting the cpm of the calibrators against their concentrations. Read the sample values directly from the curve, correcting with the dilution factor if necessary.
The spline mathematical fitting model is recommended for calibration curve. Other fitting model may give slightly different results.

Typical calibration curve (example only): this data must under no circumstances be substituted for results obtained in the laboratory.

Tube groups	Mean cpm	Concentration U/mL
Calibrator 0	96	0
Calibrator 1	1102	5
Calibrator 2	3046	10
Calibrator 3	6658	20
Calibrator 4	15109	40
Calibrator 5	30094	70
Calibrator 6	52578	180
Control 1	4252	15
Control 2	34005	80



10. METHOD LIMITATIONS

Do not extrapolate sample values beyond the last calibrator. Dilute the samples concerned and retest.

11. EXPECTED VALUES

The normal range for RIA-gnost® CA-50 was determined using 147 samples of serum from healthy men and women. Statistical evaluation demonstrated a concentration of 19 U/mL for the 90th percentile and of 25 U/mL for the 95th percentile.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Measurement range

The RIA-gnost® CA-50 kit enables concentrations of 0.4 to 180 U/mL of CA-50 to be measured.

12.2. Imprecision

This has been assessed using 2 samples assayed 20 times in the same series and in 45 different series.

Intra-assay

Samples	Mean (U/mL)	CV (%)
1	16.7	3.1
2	89.2	3.4

Inter-assay

Samples	Mean (U/mL)	CV (%)
3	11.7	5.5
4	51.8	5.1

12.3. Recovery test

Known quantities of CA-50 were added to human sera. The recovery percentages from the samples ranged from 90 to 110%.

12.4. Dilution test

Samples with high concentrations of CA-50 were diluted. The recovery percentages obtained ranged from 90 to 110%.

12.5. Specificity

The monoclonal antibodies used in this assay guarantee specific measurement of the CA-50 antigen.

12.6. Limit of detection

The limit of detection is defined as the smallest detectable concentration above 0. It has been assessed as 0.4 U/mL.

Comment

The high sensitivity of the test can only be optimum if the following instructions are complied with:
a) Avoid external contamination of the tubes.
b) Fully eliminate the entire unbound tracer fraction (by decanting or aspiration). In the event of aspiration, make sure the capillaries are not blocked. After decanting, eliminate any remaining liquid by tapping the tubes on absorbent paper.
c) Check that the measurement system and any racks used are not contaminated. Decontaminate if necessary.
d) Exclude any disruptive influences due to external radiation.

12.7. Interference

The presence of bilirubin at concentrations of up to 0.25 mg/mL, hemoglobin up to 10 g/L and triglycerides up to 20 g/L have no effect on the assay results. The immuno-assay is protected against heterophilic antibodies. However, we cannot guarantee that this protection is exhaustive.

12.8. Hook effect

No hook effect is observed for values under 20,000 IU/mL.

ASSAY FLOW CHART

Reagents / **Tubes**	**Calibrators 0 to 6 Controls Samples µL**	**Buffer µL**	Incubation Under agitation (300 rpm) 2 h 18-25°C Wash twice	**Tracer ^{125}I-anti-CA-50 µL**	Incubation Under agitation (300 rpm) 2 h 18-25°C Wash twice	Count
Calibrators	50	200		200		
Controls	50	200		200		
Samples	50	200		200		

BIBLIOGRAPHY :

Hammarström S. Chemistry and immunology of CEA, CA 19-9 and CA-50. Tumor Marker Antigens. Studentliteratur, Lund, Sweden. 1985;32-49.

Habib N, Hershman M, Haberland F, Papp L, Wood C, Williamson R. The use of CA-50 radiommunoassay in differentiating benign and malignant pancreatic disease. Br J Cancer. 1986;53:697-99.

Persson B, Ståhle E, Pahlman L, Glimelius B, et al. A clinical study of CA-50 as a tumor marker for monitoring of the colorectal cancer. Med Oncol & Tumor Pharm. 1988;5(3):165-71.

Von Rosen A, Linder S, Harmenberg U and Wiechel KL. Clinical relevance of tumour markers CA 19-9 and CA-50 in sera from patients with pancreatic duct carcinoma. Surg Oncol. 1992;1:109-13.

Van der Schouw YT, Verbeek ALM, Wobbes Th, Segers MFG and Thomas CMG. Comparison of four serum tumour markers in the diagnosis of colorectal carcinoma. Br J Cancer. 1992;66:148-54.

Collazos J, Genolla J and Ruibal A. Study of the tumor marker carbohydrate antigen 50 in liver cirrhosis. Clin Nucl Med. 1993;18:56-9.

Rakic S, Kalezic V, Grujic-Adanja G, Jagodic M. Pretreatment levels of CEA, CA 19-9, CA-50 and CA 72-4 in sera of patients with squamous cell carcinoma of the oesophagus. Eur Cancer Prev. 1993;2:36.