Décembre 2018 – Modèle 018

Cisbio Bioassays
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RIA-gnost® AFP

REF *OCFA07-AFP*

Σ 100

IVD

CE

2°C – 8°C

Trousse pour la détermination radioimmunologique de l'alphafoetoprotéine (AFP). **Pour diagnostic In Vitro** La trousse contient : Tubes revêtus Traceur ≤ **300 kBq** Calibrateurs 1 - 5 Sérum de contrôle Calibrateur 0 / Diluant Réactif de lavage Sachet plastique Notice d'utilisation **Attention:** Certains réactifs contiennent de l'azoture de sodium	**Kit for the radioimmunological determination of alpha fetoprotein (AFP).** **For In Vitro diagnostic use** Kit content : Coated tubes Tracer ≤ **300 kBq** Calibrators 1 - 5 Control serum Calibrator 0 / Diluent Wash reagent Plastic bag Instruction for use **Warning:** Some reagents contain sodium azide
	2 x 50 tubes 1 x 22 mL 5 x 0,3 mL 2 x 0,3 mL 1 x 30 mL 1 x 5 comprimés 1 1		2 x 50 tubes 1 x 22 mL 5 x 0.3 mL 2 x 0.3 mL 1 x 30 mL 1 x 5 tablets 1 1
Besteck zur radioimmunologischen Bestimmung von Alpha-Fetoprotein (AFP). **Zur In Vitro-Diagnostik** Inhalt des Kits : Teströhrchen beschichtet Tracer ≤ **300 kBq** Kalibratoren 1 – 5 Kontrollserum Null-Kalirator / Diluent Waschreagenz Plastikbeutel Gebrauchsinformation **Achtung:** Einige Reagenzien enthalten Natriumazid	**Kit per il dosaggio radioimmunologico dell'alfa-feto-proteina (AFP).** **Per uso diagnostico In Vitro** Contenuto del kit : Provette coattate Tracciante ≤ **300 kBq** Calibratori 1 – 5 Siero di controllo Calibratore 0 / Diluente Reagente di lavaggio Sacchetto di plastica Istruzioni per l'uso **Attenzione:** Alcuni reagenti contengono sodio azide
	2 x 50 Röhrchen 1 x 22 mL 5 x 0,3 mL 2 x 0,3 mL 1 x 30 mL 1 x 5 Tabletten 1 1		2 x 50 provette 1 x 22 mL 5 x 0,3 mL 2 x 0,3 mL 1 x 30 mL 1 x 5 compresse 1 1

	FRA	ENG	DEU	ITA	POL
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Wyjaśnienie symboli**
CE	Conforme aux normes européennes	European conformity	CE-Konformitäts-kennzeichnung	Conformita europea	Zgodne z normami europejskimi
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Graniczna temperatura przechowywania
LOT	N° de lot	Batch code	Chargencode	codice lotto	Numer partii
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Zużyć do
	Consulter la notice d'utilisation	Consult operating instructions	Das Handbuch zu Rate ziehen	consultare le istruzioni per l'USO	Patrz dołączona ulotka
IVD	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Diagnostyka In Vitro
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Wyprodukowane przez
REF	Référence	Catalogue number	Katalog Nr.	N. catalogo	Wzorzec
Σ 100	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Liczba probówek
CT	Tubes revêtus	Coated tubes	beschichtete Röhrchen	Provette coattate	Probówki powlekane
TRACER	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Tracciante radioattivo	Znacznik radioaktywny
CAL	Calibrateur	Calibrator	Kalibrator	Calibratore	Kalibrator
DIL CAL 0	Diluant / calibrateur 0	Diluent / Calibrator 0	Verdünnungsmittel / Kalibrator 0	Diluente / calibratore 0	Rozcieńczalnik / Kalibrator 0
CONTROL	Contrôle	Control	Kontrolle	Controllo	Kontrola
WASH	Solution de lavage	Wash solution	Waschlotion	Soluzione di lavaggio	Roztwór płuczący

MISE A JOUR / UPDATING

CE

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FRA

Modifications par rapport à la version précédente :
Mise à jour du terme Calibrateur sur page étiquette.

ENG

Changes from the previous version:
Calibrator term update on label page.

DEU

Änderungen gegenüber der Vorgängerversion:
Aktualisierung der Kalibrierbedingung auf der Labelseite.

ITA

Modifiche rispetto alla versione precedente:
Aggiornamento dei termini del calibratore sulla pagina dell'etichetta.

POL

Zmiany w stosunku do poprzedniej wersji:
Aktualizacja terminów kalibratora na stronie etykiety.

Kit for the radioimmunological determination of alpha fetoprotein (AFP).
Kit is intended for professional use.

The kit comprises:

1	vial of **^{125}I-AFP antibody** (monoclonal, mouse) < 300 kBq, 22 mL human serum with bovine albumin, rat serum, sodium azide and a red dye
2 x 50	test tubes coated with anti-AFP antibodies (monoclonal, mouse).
5	vials of AFP **calibrators**, with 0.3 mL human serum and sodium azide, concentration in the nominal range of 4 - 800 IU/mL AFP* (the calibrators are calibrated against WHO 72/225).
2	vials of AFP **control serum**, 0.3 mL human serum and sodium azide, concentration stated.
1	vial of **diluent/zero calibrator**, antigen free dilution and assay medium, 30 mL, solution with buffer, bovine albumin, sodium azide and a blue dye.
1	**wash reagent**, 5 tablets under blister.
1	plastic bag.
1	instruction for use

***** The values shown above are the target values. The real values are indicated on the label.
The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

1. Introduction

Alpha-foetoprotein (AFP) is a glycoprotein with a molecular weight of about 70000. It is formed during pregnancy in the fœtal liver and the yolk-sac. It is detectable in the embryonic serum from the sixth week of pregnancy onwards, rising to a maximum concentration between the 13th and 15th weeks of pregnancy and then decreasing to lower values until birth. After birth the AFP concentrations in the serum of the newborn infant fall very rapidly and are usually within the range of the adult values when the infant is four to six weeks old. The pattern followed by the concentrations in the amniotic fluid is similar to that in the fœtal serum (max. 14th to 16th week of pregnancy), but is lower by a factor of ≈ 100. The fœtal AFP passes into the maternal circulation from the amniotic fluid and is metabolized in the liver.
The physiological significance of AFP is not clearly understood as yet. It is thought that AFP may be a fœtal form of albumin.
The maternal serum concentration of AFP increases during pregnancy, reaching its peak during the last trimester (34th-35th week of pregnancy).

2. Clinical results with RIA-gnost®AFP

2.1. Clinical significance of quantitative measurement of AFP

2.1.1 Diagnosis of AFP-producing tumours in the testes

As circulating AFP has a half-life of between four and five days, it is usually possible, just two to three weeks after the surgical removal of an AFP-producing tumour, to determine whether it has been completely excised and whether there are any metastases. If an AFP-producing tumour has been totally removed without metastases, the serum AFP concentration falls to the normal range. After surgery it is advisable first to carry out a screening check so as to detectany increase in serum AFP at an early stage. It is not so much an increase in serum AFP to above a specific limit that governs later therapy, as a continuous rise within the observation period. It is best to measure the seric hCG at the same time.

2.1.2 Serum AFP in liver disorders

Raised serum AFP concentrations are also found in patients with liver disorders such as hepatitis, cirrhosis of the liver and liver cell carcinoma.

2.1.3 Screening during pregnancy

Measurement of the AFP concentration in maternal serum between the 14th and 21st week of pregnancy is carried out mainly as a screening test for the presence of an open neural tube defect or an anencephaly.
AFP serum concentrations that are higher than the calibrator level for the stage of pregnancy in question can indicate the presence of conditions that put the fœtus at risk, in some cases causing its death in utero, such as spina bifida or anencephaly, or even in some cases omphalocele and congenital nephrosis. Other causes can be hepatic disease in the mother or multiple pregnancy. Raised AFP levels are also encountered when the stage of pregnancy has not been correctly determined. This parameter must be taken into account when measuring AFP.
If the serum AFP level still exceeds the 2.5-fold median when the stage of pregnancy has been correctly determined and the test has been repeated, and the ultrasound examination does not suggest any other reason for raised serum AFP concentration, it is advisable to measure the AFP concentration in the amniotic fluid obtained by amniocentesis. These samples are diluted with the diluent so that they come within the measuring range.

2.2 Normal values

2.2.1 Tumour diagnosis

The upper limit of the normal range has been determined by n = 130 serum samples from healthy men and n = 168 serum samples from healthy, non-pregnant women using the 95th percentile. An upper limit of 5 IU AFP/mL results for both groups.
Seric AFP values above these limits can be regarded as an indication of a tumour. The AFP tumour marker is particularly suitable for the follow-up of patients with AFP-producing tumours.

2.2.2 Screening during pregnancy

The determination of the normal range for RIA-gnost®AFP was carried out by measuring 12 264 sera of normal pregnancies between the 15th and 21st current weeks of pregnancy. The median (50th percentile) and the 2.5-fold median were calculated to establish the normal range (see Table 1).

Table 1 : Calibrator values for maternal serum AFP concentrations between the 14th and 21st week of pregnancy*

Weeks of gestation Completed Current	14th 15th	15th 16th	16th 17th	17th 18th	18th 19th	19th 20h	20th 21st
n	116	1587	4112	3092	2038	1065	254
Median (IU/mL)	21	27.5	31	37	42	48	56.5
2.5-fold Median (IU/mL)	52.5	69	77.5	92.5	105	120	141

*The definition of gestational age varies for different countries

The range given in Table 2 was determined for the AFP concentration in the amniotic fluid in normal pregnancy with RIA-gnost®AFP after appropriate dilution. It is important to ensure that the amniotic fluid sample is free from fœtal erythrocytes.

Table 2 : AFP concentration in the amniotic fluid of women between 15th and 21st week of pregnancy

Current week of pregnancy	15th	16th	17th	18th	19th	20th	21st
n	61	119	154	88	49	43	38
Median (IU/mL)	10620	9440	8220	7750	5310	5530	4780
3-fold Median (IU/mL)	31860	28320	24660	23250	15930	16590	14340

It is recommended to control the calibrator values in your own laboratory.

3. Principle of measurement and characteristic data of the RIA-gnost® AFP

3.1 Principle

RIA-gnost® AFP enables the in vitro determination of alpha-fetoprotein in human serum (or plasma) and amniotic fluid by the "sandwich" assay principle. A complex of anti-AFP antibodies (monoclonal, mouse) bound to the tube wall, AFP in the sample and ^{125}I-labelled anti-AFP antibodies (monoclonal, mouse) are formed during this process.
The amount of tracer specifically bound to the coated test tubes is measured with a gamma counter.
The evaluation of the unknown samples is carried out by reading from a calibrator curve constructed under identical conditions.
All the samples outside the measuring range, as well as all amniotic fluid samples, can be diluted down to 1:100 with the diluent contained in the kit.
The monoclonal antibodies used in the kit are highly specific for AFP. There is virtually no risk of a cross reaction with other serum proteins occurring in the physiologically relevant concentration ranges.

3.2 Specific characteristics of the assay

3.2.1 Imprecision

The table below presents the inter-assay (between-run) variation for the kit RIA-gnost® AFP and was determined using 3 samples measured in 20 different runs in triplicate. The intra-assay (within-run) variation was determined by 30 measurements of 3 samples.

Between-run

	Mean Concentration AFP (IU/mL)	%CV
SAMPLE 1	9.28 +/- 0.49	5.3%
SAMPLE 2	60.60 +/- 1.72	2.8%
SAMPLE 3	246.58 +/- 14.42	5.8%

Within-run

	Mean Concentration AFP (IU/mL)	%CV
SAMPLE 4	10.19 +/- 0.43	4.3 %
SAMPLE 5	63.03 +/- 1.91	3.0 %
SAMPLE 6	273.30 +/- 14.51	5.3 %

3.2.2 Detection limit

The detection limit is defined as being the smallest concentration different from 0 with a confidence interval of 95%. It has been determined as being 0.3 IU/mL.

3.2.3 Dilution test

3 serum samples of different concentrations were diluted in DIL/CAL0 and assayed in 3 replicates. Recovery percentages obtained range from 80 to 120% with a dilution factor not exceeding 1/8.

Samples	Dilution factor	Measured (IU/mL)	Expected (IU/mL)	Dilution Recovery%
Sample 1 (730.21IU/mL)	1	730.2	730.2	100.0%
	1/2	390.7	365.1	107.0%
	1/4	186.4	182.5	102.1%
	1/8	101.3	91.3	111.0%

Samples	Dilution factor	Measured (IU/mL)	Expected (IU/mL)	Dilution Recovery%
Sample 2 (2873.1IU/mL)	1 (after a first dilution 1/10)	287.3	287.3	100.0%
	1/2	140.5	143.6	97.8%
	1/4	74.3	71.8	103.5%
	1/8	42.3	35.9	117.9%

Samples	Dilution factor	Measured (IU/mL)	Expected (IU/mL)	Dilution Recovery%
Sample 3 (8144.6IU/mL)	1 (after a first dilution 1/20)	407.2	407.2	100.0%
	1/2	184.9	203.6	90.8%
	1/4	95.7	101.8	94.0%
	1/8	57.5	50.9	112.9%

3.2.4 Recovery test

Calibrators 0 to 5 were mixed with a ratio of 1:1 to 2 serum samples with various initial Alpha-foetoprotein concentrations (level 1 and level 2). Each sample (non-spiked and spiked) was assayed in triplicates in one run. Alpha-foetoprotein concentrations were measured, Mean Antigen recovery percentages ranged between 90-110% of the expected values.

3.2.5. Interference

The presence of bilirubin at concentrations of up to 500 mg/L, hemoglobin up to 5 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.

4. Working procedure

4.1. Equipment required

Precision micropipettes or similar with disposable tips, permitting the dispensing of 50 and 200 µL, measuring cylinders, horizontal shakers, gamma scintillator counter calibrated for 125 iodine measurement.

4.2. Preparation of the reagents

Do not mix reagents from different lots.

The kit components, which have been stored at +2 et +8°C, are brought up to room temperature (18 - 25°C) before use. The washing buffer is prepared by dissolving five buffer tablets in 500 mL distilled water.

All unused reagents should be stored at 2-8°C. The remaining test tubes are stored in the original package, resealed.

4.3. Preparation of the serum samples

When blood samples have been taken, serum or plasma is obtained by the usual methods. The serum or plasma is used directly in the assay or stored for up to 3 days at 2-8 °C. If stored for a longer period, this should be at -20 °C. The serum samples must be carefully mixed after thawing.

4.4. Warnings and Precautions

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HVC antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit 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.

4.5. Assay procedure (see Table 3)

1. Number sufficient coated test tubes as shown in Table 3 (1 diluent D = CAL_0, 5 calibrators CAL_1 - CAL_5, 2 control sera C_1, C_2, up to 42 serum samples).
2. Pipette 50 µL calibrator (or patient sample) into the bottom of the coated tubes. Use a new pipette tip for each sample.
3. Dispense 200 µL diluent into each test tube.
   Stages 2 and 3 can be carried out automatically using a dispenser/dilutor system.
4. Shake the test tubes on a horizontal shaker for 15 minutes (300 ± 50 rpm) at 18 - 25°C.
5. Then introduce 1 mL washing buffer into each test tube, decant (aspirate) and wash again with 1 mL.
6. Dispense 200 µL ^{125}I-anti-AFP solution into the bottom of each incubation tube.
7. Shake for 15 minutes (300 ± 50rpm) at 18-25°C.
8. Wash, as described in 5.
9. Measure the tubes for 1 minute in the gamma scintillation counter.

Note

It is important to ensure that this procedure is carried out rapidly (e. g. rack by rack). The total batch assayed should not exceed 200 tubes.

The high sensitivity of the assay can only be achieved if the following recommendations are followed:

a) Avoid external contamination of the test tubes.
b) Ensure that the unbound tracer fraction is completely removed (decantation/aspiration). During aspiration the capillary tubes must not become blocked; after decantation tap the test tubes well onto an absorbent surface.
c) Check the measuring device and any associated equipment that may be used regularly, and if necessary decontaminate.
d) Exclude any interference effect from external sources of radiation.

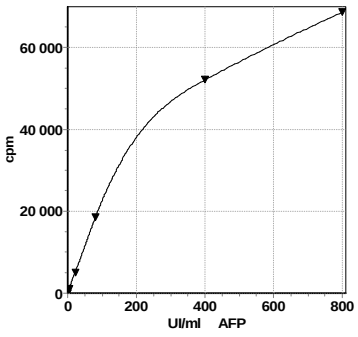
4.6. Evaluation of the results

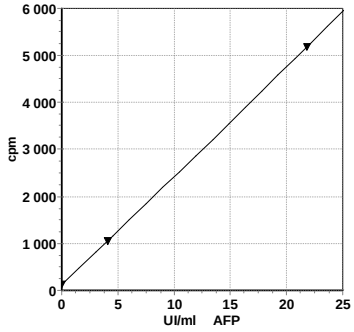
For each group of tubes, calculate the mean counts after subtracting the background.
Draw up the calibrator curve by plotting the calibrator's cpm against their concentrations.
Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.

The spline mathematical fitting model is recommended for calibration curve. Other fitting model may give slightly different results.

Example of a calibrator curve

Group of tubes	cpm mean	Concentration IU/mL
Calibrator 0	128	0
Calibrator 1	1060	4.1
Calibrator 2	5181	21.8
Calibrator 3	18640	81.0
Calibrator 4	52155	400.0
Calibrator 5	68716	800.0
Control 1	2540	10.5
Control 2	22122	101.0





5. Radioprotection 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 or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate safety.
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.

Table. 3 : AFP assay procedure

	Calibrators (µL)						Control sera (µL)		Samples (µL)		
Labelling of the tubes	CAL_0 (diluent)	CAL_1	CAL_2	CAL_3	CAL_4	CAL_5	C_1	C_2	1	2	etc.
Calibrator S0 = Diluent	50/50										
CAL_1		50/50									
CAL_2			50/50								
CAL_3				50/50							
CAL_4					50/50						
CAL_5						50/50					
Control sera C1							50/50				
C2								50/50			
Samples									50	50	etc.
Diluent	◄---------- 200 µL ----------►										
	Shake for 15 minutes (300 ± 50 rpm)										
Washing buffer	◄---------- 1 mL ----------►										
	Decant (aspirate); wash with 1 mL										
Anti-AFP tracer	◄---------- 200 µL ----------►										
	Shake for 15 minutes (300 ± 50 rpm)										
Washing buffer	◄---------- 1 mL ----------►										
	Decant (aspirate); wash with 1 mL										
	Measure										

BIBLIOGRAPHY:

Ganiats TG, Berry CC, Fullerton JT. Effect of normal MSAFP screening on maternal age for genetic amniocentesis. J Clin Epidemiol. 1990;43/11:1143-8.

Haddow JE. Prenatal screening for open neural tube defects, Down's Syndrome, and other major fetal disorders. Semin Perinatol. 1990;6:488-503.

Jalanko H. Alpha-fetoprotein in cancer. Ann Chir Gynaecol. 1989;78:27-31.

Javadpour N. The role of biologic tumor makers in testicular cancer. Cancer. 1980;45:1755.

Nomura F, Ohnishi K, Tanabe Y. Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. Cancer. 1989;64:1700-7.

O'Brien WF, Knuppel RA, Torres C, Sternlicht D. Potential prenatal predictions of Down syndrome : a statistical analysis. Am J Obstet Gynecol. 1990;163:1796-8.

Rimbaud C, Hoffenbach A, Rudant C, et al. Tyrosinemia and hepatoma importance of the AFP follow-up to detect the malignant degeneration. In Protides of the Biological Fluids. 1979. ED Dr H. PEETERS. Pergamon Press Oxford and New-York.

Second report of the U.K. Collaborative Study on Alpha-fetoprotein relation to neural-tube defects. Lancet II. 1979;651.

Spencer K, Coombes EJ, Mallard AS, Ward AM. Free beta human choriogonadotropin in Down's syndrome screening : a multicentre study of its role compared with other biochemical markers. Ann Clin Biochem. 1992;29:506-18.

Tatarinov VS. Production of embryo-specific alpha-globulin in the blood sera of patients with primary liver tumor. Vopr Med Khim. 1964;10:90-1.

Uriel J. The physiological role of Alpha-Fetoprotein in cell growth differentiation. J Nucl Med Allied Sci. 1979;33/3:12-7.

Uriel J, Trojan J, Dubouch P, et al. Intracellular alpha-fetoprotein and albumin in the developing nervous system of the baboon. Path Biol. 1982;30:79-83.