Cisbio Bioassays
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AFP

REF *ELSA2-AFP*

Σ 96

IVD CE

2°C – 8°C

Trousse pour le dosage immunoradiométrique de l'alpha-foetoprotéine humaine dans le sérum, le plasma ou le liquide amniotique. **Pour diagnostic In Vitro** La trousse contient : ELSA 4 x 24 tubes Traceur ≤ **277,5 kBq** 1 x 30 mL Calibrateur 0 1 x 10 mL Calibrateurs 1 – 6 6 x 0,5 mL Contrôle 1 x 0,5 mL Tampon 1 x 31 mL Sachet plastique 1 Notice d'utilisation 1 **Attention:** Certains réactifs contiennent de l'azoture de sodium	**Kit for the immunoradiometric assay for a direct quantitative determination of human alpha-foetoprotein in serum, plasma or amniotic fluid.** **For In Vitro diagnostic use** Kit content : ELSA 4 x 24 tubes Tracer ≤ **277.5 kBq** 1 x 30 mL Calibrator 0 1 x 10 mL Calibrators 1 – 6 6 x 0.5 mL Control 1 x 0.5 mL Buffer 1 x 31 mL Plastic bag 1 Instruction for use 1 **Warning:** Some reagents contain sodium azide	**Immunoradiometrischer Test zur Bestimmung von humanem Alpha-Fetoprotein inSerum, Plasma oder Fruchtwasser.** **Zur In Vitro-Diagnostik** Inhalt des Kits : ELSA 4 x 24 Röhrchen Tracer ≤ **277,5 kBq** 1 x 30 mL Kalibrator 0 1 x 10 mL Kalibratoren 1 – 6 6 x 0,5 mL Kontrolle 1 x 0,5 mL Puffer 1 x 31 mL Plastikbeutel 1 Gebrauchsinformation 1 **Achtung:** Einige Reagenzien enthalten Natriumazid
Kit per il dosaggio immunoradiometrico dell'alfa-feto-proteina umana nel siero, nel plasma o nel liquido amniotico. **Per uso diagnostico In Vitro** Contenuto del kit : ELSA 4 x 24 provette Tracciante ≤ **277,5 kBq** 1 x 30 mL Calibratore 0 1 x 10 mL Calibratori 1 – 6 6 x 0,5 mL Controllo 1 x 0,5 mL Tampone 1 x 31 mL Sacchetto di plastica 1 Istruzioni per l'uso 1 **Attenzione:** Alcuni reagenti contengono sodio azide	**Equipo para la determinación inmunorradiométrica de alfafetoproteina humana en suero, plasma o líquido amniótico.** **Para uso de diagnóstico In Vitro** Contenido del equipo : ELSA 4 x 24 tubos Trazador ≤ **277,5 kBq** 1 x 30 mL Calibrador 0 1 x 10 mL Calibradores 1 – 6 6 x 0,5 mL Control 1 x 0,5 mL Tampón 1 x 31 mL Bolsa de plástico 1 Instrucciones de uso 1 **Precauciones:** Algunos reactivos contienen azida sódica	**Δοκιμασία για τον ραδιοανοσολογικό προσδιορισμό της ανθρώπινης αλφα-φετοπρωτεΐνης στον ορό, το πλάσμα ή το αμνιακό υγρό.** **Για διαγνωστική χρήση in vitro** Περιεχόμενα της τυποποιημένης συσκευασίας ELSA 4 x 24 σωλήνων Ιχνηθέτης ≤ **277,5 kBq** 1 x 30 mL Βαθμονομητή 0 1 x 10 mL Βαθμονομητές 1 – 6 6 x 0,5 mL Μάρτυς 1 x 0,5 mL Ρυθμιστικό διάλυμα 1 x 31 mL Πλαστική θήκη 1 Οδηγίες χρήσεως 1 **Προσοχή:** Ορισμένα αντιδραστήρια περιέχουν αζίδιο νατρίου

	FRA	ENG	DEU	ITA	SPA	ELL	BUL	RUS
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Significado de los simbolos**	**Επεξήγηση των συμβόλων που**	**Обяснение на символите**	**Объяснение символов**
CE	Conforme aux normes européennes	European conformity	CE-Konformitäts-kennzeichnung	Conformita europea	Conformidad europea	European conformity	Европейската съответствието	Европейское соответствие
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Limites de temperatura de almacenamiento	Περιορισμός θερμοκρασίας φύλαξης	Ограничаване на температурата на съхранение	Ограничение температуры хранения
LOT	N° de lot	Batch code	Chargencode	codice lotto	Código de lote	Κωδικός παρτίδας	номер	номер партии
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Consumir antes de	Ημερομ. λήξης	Срок на валидност	дата истечения срока действия
	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	Консултирайте инструкции за работа	Учитывать Руководство по эксплуатации
IVD	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Dispositivo de diagnóstico In Vitro	διαγνωστική συσκευή In Vitro	За ин витро диагностика устройство	In Vitro диагностическое устройство
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Fabricado por	Κατασκευάζεται από την	Произведено от	Изготовитель
REF	Référence	Catalogue number	Katalog Nr.	N. catalogo	Número de catálogo	Αριθμός καταλόγου	Каталожен номер	номер по каталогу
	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Número de determinaciones	Αριθμός προσδιορισμών	Брой определяния	Количество определений
CT	Tubes revêtus	Coated tubes	beschichtete Röhrchen	Provette coattate	Tubos recubiertos	Επιστρωμένα σωληνάρια	Покритите тръби	пробирки с покрытием
TRACER	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Tracciante radioattivo	Trazador radiactivo	Ραδιενεργός ιχνηθέτης	Индикатор	пробирки с покрытием
CAL	Calibrateur	Calibrator	Kalibrator	Calibratore	Calibrador	Βαθμονομητής	Калибратор	калибратор
CONTROL	Contrôle	Control	Kontrolle	Controllo	Control	Ορός ελέγχου	Контрол	Управление
BUF	Tampon	Buffer	Puffer	Tampone	Tampon	εξουδετερώτης	буфер	буфер

MISE A JOUR / UPDATING

CE

ELSA2-AFP
Cisbio Bioassays – Février 2017 - Modèle 29

FRA

Modifications par rapport à la version précédente :
Nouveau logo /1. Ajout de „La trousse est destinée à un usage professionnel“ /7.2 essais en double pour les calibrateurs et le contrôle / 9. Information sur le mode de lissage.

ENG

Changes from the previous version:
New logo /1."The kit is intended for professional use" adding / 7.2 assays in duplicate for calibrators and control / 9. Information on fitting model.

DEU

Änderungen gegenüber der Vorgängerversion:
Neues logo /1. „Das Kit ist für den professionellen Gebrauch vorgesehen“ hinzufügen / 7.2 Tests in Doppelbestimmung für Kalibratoren und Kontrolle / 9. Informationen zum Funktionsmodell.

ITA

Modifiche rispetto alla versione precedente:
Nuovo logo /1. Aggiunto "Il kit è destinato all'uso professionale" / 7.2 dosaggi in doppio per calibratori e controlli. / 9. Informazioni sul modello di fitting

SPA

Cambios desde la versión anterior:
Nuevo logo / 1. Se ha añadido “El kit está destinado a uso profesional” / 7.2 ensayos por duplicado para los calibradores y el control. / 9. información sobre el modelo de ajuste

ELL

Αλλαγές από την προηγούμενη έκδοση:
νέο λογότυπο /1. Προσθήκη "Το κιτ προορίζεται για επαγγελματική χρήση"/ 7.2 Προσθήκη βαθμονομητών και ορών ελέγχου για τη δοκιμασία εις διπλούν / 9. Πληροφορίες σχετικά με το μοντέλο προσαρμογής

BUL

Промени от предишната версия :
ново лого /1.Добавено „Комплектът е предназначен за професионална употреба“ /7.2 дублирани анализи за калибраторите, контролите /9. зирана в съответствие с английската версия информация относно апроксимиращия модел

RUS

Изменения по сравнению с предыдущей версией:
новый логотип /1. добавлено «Набор предназначен для профессионального использования» / 7.2 анализы в двух экземплярах для калибраторов, контрольных образцов / 9. информация о модели подбора

1. NAME AND INTENDED USE

ELSA2-AFP is an immunoradiometric assay for a direct quantitative determination of human alpha-fetoprotein in serum, plasma or amniotic fluid.
The kit is intended for professional use.

2. INTRODUCTION

Alpha-foetoprotein (AFP) is a 70,000 D glycoprotein, synthesized by both fetal liver and yolk sac cells. It is the major fetal component with its maximum level reaching 3 mg/mL around the 13th week of pregnancy. In amniotic fluid, the maximum level is about 20 - 50 µg/mL in the 15th week, and in maternal serum the maximum is in the 34th week (200 ng/mL). The newborn AFP serum level rapidly decreases during the first year down to an adult serum level (less than 15 ng/mL). The biological role of AFP remains unclear, but it seems to be involved in the transporting of fatty acids (especially the unsaturated).
At present, AFP assay is particularly useful in the following cases:

- In cancerology, AFP is useful for diagnosis and permits post-therapeutic follow-up of:
  . hepatocellular carcinomas, particularly those following liver cirrhosis,
  . all teratocarcinomas, particularly of the testes and ovaries,
  . liver metastases originating from different cancers, especially those of the digestive tract.
- In hepatology, AFP is an indicator of intense liver regeneration in viral hepatitis.
- In pediatrics, AFP assay can be used to differentiate between biliary atresia and neonatal hepatitis and to confirm hereditary tyrosinosis.
- In obstetrics, AFP measurement can be used to diagnose fetal neural tube defects (anencephaly, *spina bifida*) in maternal serum and amniotic fluid samples taken between 16-18 weeks of pregnancy. Moreover, an increased AFP level can indicate fetal distress or multiple pregnancy, and a decreased level can suggest toxemia, slow fetal growth or a placental tumor. An accurate interpretation of AFP levels needs to take real gestational age into account.

3. PRINCIPLE

ELSA2-AFP is a solid phase two-site immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote antigenic sites on the AFP molecule. The first is coated onto the ELSA solid phase, while the second, radiolabelled with iodine 125, is used as a tracer.
The AFP molecules present in the calibrators or the samples to be tested are "sandwiched" between the two antibodies. Following the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.
The radioactivity bound to the ELSA is proportional to the concentration of AFP present in the sample.

4. REAGENTS

Each kit contains enough reagents for 96 tubes. The expiry date is marked on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
ELSA: ready for use. Anti-AFP monoclonal antibody coated on ELSA fixed to the bottom of the tube.	CT	4 traypacks of 24 tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.
ANTI-AFP ^{125}I: ready for use. ^{125}I anti-AFP monoclonal antibody, buffer, sheep serum, sodium azide, non-immunized mouse immunoglobulins. ≤ 277.5 kBq (≤ 7.5 µCi).	TRACER	1 30 mL vial	2-8°C until the expiry date. After opening and first use, 15 days at 2-8°C.
CALIBRATOR 0: ready for use. Calf serum, sodium azide.	CAL	1 10 mL vial	2-8°C until the expiry date. After opening and first use, 15 days at 2-8°C.
CALIBRATORS: ready for use. Highly purified AFP (human), calf serum, sodium azide. 3 - 30 - 100 - 250 - 500 - 800 ng/mL.*	CAL	6 0.5 mL vials	2-8°C until the expiry date. After opening and first use, 15 days at 2-8°C.
CONTROL: ready for use. Highly purified AFP (human)**, calf serum, sodium azide.	CONTROL	1 0.5 mL vial	2-8°C until the expiry date.
BUFFER: ready for use. Buffer, sodium azide, non-immunized mouse immunoglobulins.	BUF	1 31 mL vial	2-8°C until the expiry date.
PLASTIC BAG		1	

* The values shown above are only target values; the true value of each calibrator is shown on its label. 1 ng CIS = 1 IU 1st IS 72/225.
** The acceptance range true values are printed on the vial label.

5. PRECAUTIONS FOR USE

5.1. Safety measures

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-HCV 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.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method for doing this 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. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic 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 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 microbic contamination of the reagents or of the water used for washing. Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum, plasma or amniotic fluid. If the assay is performed within 24 hours, the samples should be kept at 2-8°C. Otherwise, they should be divided into aliquots and stored deep frozen (-20°C).

Dilution

Should elevated AFP levels be suspected, the dilution is performed with the calibrator 0 found in the kit. It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar with disposable tips, capable of dispensing 50 µL and 300 µL. Their calibration should be checked regularly.
Distilled water. Disposable plastic tubes. Vortex-type mixer. Circular horizontal shaker. Gamma scintillation counter calibrated for 125 iodine measurement.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing the reagent into the ELSA tubes is also carried out at room temperature (18-25°C).
The assay requires the following groups of tubes: O calibrator group for the determination of non-specific binding, Calibrator groups to establish the calibrator curve,Control group for the control, Sx groups for the samples to be assayed. It is recommended that the assay be performed in duplicate for the calibrators, the control and the samples.
Respect the order in which reagents are to be added:
Dispense 300 µL of buffer solution into all ELSA tubes.
Add 50 µL of calibrators, control or samples into the corresponding groups of tubes.
Gently mix each tube with a vortex type mixer.
Incubate for 30 minutes at room temperature (18-25°C) under shaking (400 rpm).

Wash the ELSA tubes as follows:
Aspirate the content of the tubes as completely as possible.
Add 3.0 mL of distilled water to each tube before emptying them again.
Repeat the process once.
To obtain reliable and reproducible results, the different washing steps have to be performed correctly. As much as possible of the incubation and washing solutions must be removed. If the washing is carried out manually, the tip of the aspirating device must be placed right at the bottom of the ELSA tube.
Dispense 300 µL of ^{125}I anti-AFP monoclonal antibody into each ELSA tube.
Incubate for 30 minutes at room temperature (18-25°C) under shaking (400 rpm).
Wash the ELSA tubes as previously described.
Measure the radioactivity bound to the ELSA with a gamma scintillation counter.

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 specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

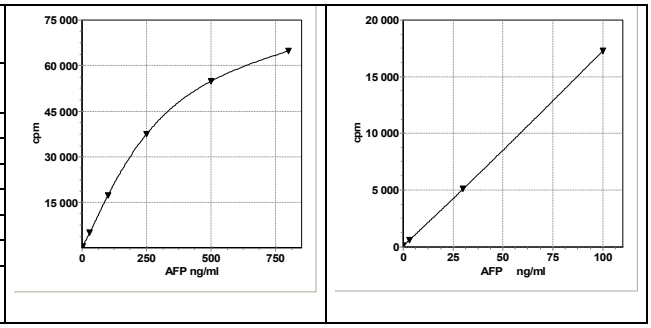
9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background. Draw up the calibrator curve by plotting the calibrators' 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.

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

Tube Groups	Mean cpm	Concentration ng/mL
Calibrator 0	110	0
Calibrator 1	603	3
Calibrator 2	5112	30
Calibrator 3	17281	100
Calibrator 4	37504	250
Calibrator 5	54872	500
Calibrator 6	64852	800
Control	1754	10



10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.
Do not extrapolate sample values beyond the last calibrator. Dilute the samples concerned and re-assay.

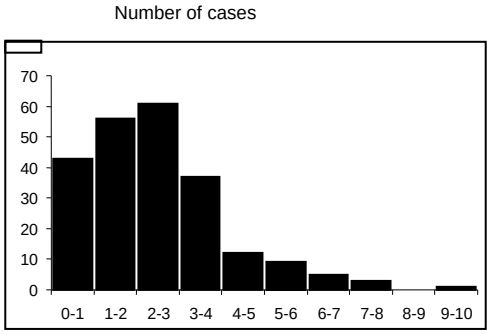
11. EXPECTED VALUES

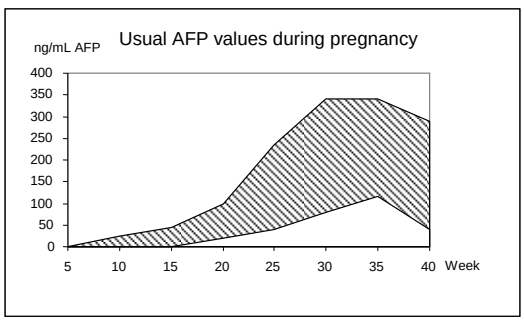
The values given below are only indicative and it is recommended for each laboratory to establish its own normal range.

Distribution of normal values

These values have been obtained on presumably healthy subjects from both sexes

n = 220
x = 2,55 ng/mL
D.S =1,58 ng/mL
96,4 % ≤ 6 ng/mL





12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 3 samples with different concentrations. They were tested either 30 times in the same series of assays or in duplicate in 15 different series.

Samples	X ng/mL	Intra-run CV %
1	26.9	4.0
2	222	4.8
3	437	5.3

Samples	X ng/mL	Inter-run CV %
4	27.0	4.9
5	244	4.2
6	460	6.5

12.2. Recovery test

Known quantities of AFP were added to human sera. The recovery percentages of AFP in the samples ranged from 97.7 to 101.7%.

12.3. Specificity

The antibodies used in this assay guarantee a measurement which is completely specific for AFP.

12.4. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95%. It has been assessed as being 0.5 ng/mL.

12.5. Interference

No interference with bilirubin, haemoglobin, and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L, and 20 g/L, has been observed.
The immunoassay is protected against any human anti-mouse antibody (HAMA) interference by the addition of a protector to the tracer (non-specific mouse immunoglobulins). However, we can not guarantee that this protection is exhaustive.

ASSAY FLOW CHART

Tubes	Buffer µL	Calibrators Control Samples µL	Incubate for 30 minutes at 18-25°C under shaking Wash twice with distilled water	^{125}I anti-AFP µL	Incubate for 30 minutes at 18-25°C under shaking Wash twice with distilled water	Count
Calibrators	300	50		300		
Control	300	50		300		
Samples	300	50		300		

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INTERACTION IS EVERYTHING