RK-800CTACE180919

AFP [I-125] IRMA KIT

(REF: RK-800CT)

The ^{125}I-AFP IRMA system provides direct quantitative *in vitro* determination of human alpha-foetoprotein (AFP) in human serum. AFP can be assayed in the range of 0-500 IU/ml using 50 µl serum samples.

Introduction

Alpha-foetoprotein (AFP) is a glycoprotein with a molecular mass of 65000. It is normally produced in large amounts by the foetal liver. AFP level increases progressively and reaches a peak at the 30th week in the maternal serum, thereafter, it decreases gradually. An elevated maternal serum AFP is associated with neural tube defect (spina bifida) and placental abnormalities, whereas the decreased AFP levels in both maternal serum and amniotic fluid are related to foetal chromosomal abnormalities.

Patients affected by liver carcinoma, testicular or ovarian teratocarcinoma commonly present high AFP levels. Although less frequently, an increased AFP concentration may also be observed in hepatitis, gastric, breast and bronchial tumours.

This kit can be used in the risk assessment of Down's Syndrome (Trisomy 21) in combination with other biochemical and ultrasound parameters as specified above, taking also into account other data like maternal age and weight and using a validated software for Down's Syndrome risk assessment (***see Annex***):

1. **Triple test**: maternal serum AFP, hCG and unconjugated Estriol determination in the second trimester of pregnancy.
2. **Quadruple test**: maternal serum AFP, hCG, unconjugated Estriol and Inhibin-A determination in the second trimester of pregnancy.
3. **Integrated test**: maternal serum PAPP-A determination and nuchal translucency (NT) thickness measurement by an ultrasound scan in the first trimester of pregnancy and maternal serum AFP, hCG, unconjugated Estriol and Inhibin-A determination in the second trimester.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the AFP molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 2-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of AFP, the unknown concentration of AFP in patient samples can determined.

Contents of the kit

1. 1 bottle of TRACER (21 ml), ready to use, containing about 740 kBq ^{125}I-anti-AFP and capture anti-AFP antibody in buffer with red dye and 0.1 % NaN_3.

2. 6 vials of STANDARDS (1 x 3.0 ml, 5 x 0.5 ml), containing (S1-S6) 0, 2, 10, 40, 150, 500 IU/ml AFP (calibrated to WHO 1st IRP 72/225) in bovine serum with 0.1% NaN_3. (1 IU/ml = 1.21 ng/ml)

3. 1 vial of CONTROL SERUM. 1.0 ml human serum with 0.1% NaN_3. The concentration of the control serum is specified in the quality certificate enclosed.

4. 2 boxes of COATED TUBES, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

5. 1 bottle of WASH BUFFER CONCENTRATE (20 ml), containing 0.2% NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter

Recommended tools and equipment

repeating pipettes, dispenser with 1-L reservoir (instead of the 2-ml pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Hemolyzed and lipemic specimens may give false values and should not be used. Repeated freezing and thawing of specimens must be avoided

If in an initial assay the serum sample is found to contain more than 500 IU/ml AFP, the sample can be diluted 10-fold with S1 standard and reassayed as described in Assay Procedure.

Preparation of reagents, storage

Add the wash buffer concentrate (20 ml) to 700 ml distilled water to obtain 720 ml wash solution. Upon dilution store at 2-8°C until expiry date of the KIT.

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date of the KIT. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide , refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S1-S6), control serum and samples.
2. Homogenize all reagents and samples by gentle mixing to avoid foaming.
3. Pipette 50 µl of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
4. Pipette 200 µl of tracer into each tube.
5. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
6. Incubate tubes for 2 hours, shaking at room temperature.
7. Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
8. Return the tube-rack to an upright position, and repeat step-7 two more times.
9. Count each tube for at least 60 seconds in a gamma counter and calculate the AFP concentrations of the samples as described in calculation of results.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**50**		
Control			**50**	
Sample				**50**
Tracer	**200**	**200**	**200**	**200**
Shake for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{2\text{-}6} / C / M_x\ (cpm) - S_1(cpm)}{T(cpm)} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of AFP.

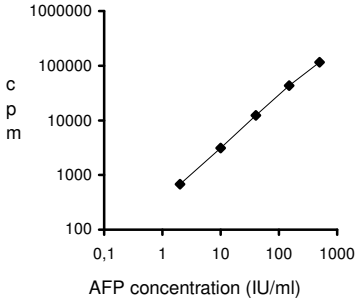

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples!)*

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	B/T%
T	257181 256904	257042	-
S1	65 87	76	0.03
S2	771 747	759	0.27
S3	3192 3221	3207	1.22
S4	12423 12282	12353	4.78
S5	44624 42117	43371	16.84
S6	116753 113293	115023	44.72
C	10683 10208	10445	4.03

Determine the AFP concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.
Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used. Automated data processing systems are also available.

Characterization of assay

Sensitivity

For the analytical sensitivity 0.06 IU/ml has been obtained by assaying 15 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Hook effect

There is no high dose " hook effect " up to the AFP concentration of 8500 IU/ml .

Specificity

The monoclonal antibodies used in this IRMA kit are specific for AFP. No cross reactivity was found with human albumin.

Precision and reproducibility

8 control samples were assayed in 15 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 15 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (IU/ml)	CV %	Mean (IU/ml)	CV %
8.04	3.2	7.46	3.9
14.90	3.0	15.21	3.1
24.97	2.8	25.21	2.3
32.07	2.1	31.79	4.6
47.49	2.2	49.38	3.3
55.71	2.7	57.87	3.6
69.34	3.3	71.85	2.8
177.45	2.4	178.64	4.0

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of AFP. The average per cent recovery for 3 serum pools spiked with AFP at 5 levels each was 98.13 % with a range of 92.37 % to 104.39 %.

Dilution test (linearity)

5 samples were measured in a series of dilution with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$\mathbf{y} = 1.0645\mathbf{x} + 0.0217 \quad R^2 = 0{,}998 \quad n = 25$

Expected Values

It is recommended that each laboratory determine a reference range for its own patient population, since this may vary in different laboratories or regions.

Healthy male: 0.47 – 5.39 IU/ml.
Healthy female: 0.37 – 4.41 IU/ml

In the second trimester of pregnancy:

Pregnancy Week	N	1 MOM (IU/ml)
14	22	23.25
15	1107	25.7
16	1019	29.1
17	137	32.4

Every three months MoM values should be checked and, if necessary, recalculated.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 67.5 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C.
Shelf-life: 60 days from availability.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	2 8°C	Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material
REF	Catalogue number		

CE 1011

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Annex to the Instructions for Use

In vitro kits for Down's Syndrome risk assessment

This kit has been validated for the risk assessment of 21 Trisomy, using the following kits and software:

Kit / software name	Code	Manufacturer
PAPP-A IRMA	RK-4 CT	Institute of Isotopes Ltd.
Free βhCG IRMA	RK-820CT	Institute of Isotopes Ltd.
AFP IRMA	RK-800 CT	Institute of Isotopes Ltd.
hCG RIA	RK-770 CT	Institute of Isotopes Ltd.
Unconjugated Estriol RIA	RK-3 CT	Institute of Isotopes Ltd.
Active Inhibin-A ELISA	DSL-10-28100	DSL - Beckman Coulter
αlpha - Antenatal Screening Software for Down's Syndrome and Neural Tube Defects.		Logical Medical Systems Ltd.
*The kits and software listed before are CE marked. They can be used together for the risk assessment of Trisomy 21, according to the 98/79 EC IVD directive and based on the conformity assessment performed by an authorized notified body (**CE1011**).*		

Resumed results of validation, regarding efficiency of risk assessment:

Sensitivity

Screening method	Number of tests performed	Positive cases with the test		Cases confirmed by cytogenetic test		Fals positive cases	
		No.	%	No.	% of positive tests	No.	%
Combined Test	1389	42	3,02	2	4,76	40	2,88
Quadruple Test	539	30	5,57	1	3,33	29	5,38
Integrated Test	1741	47	2,70	3	6,38	44	2,53

Specificity

Screening method	Number of tests performed	Negative cases	Negative cases with the test		Fals negative cases
			No.	%	No. (%)
Combined Test	1389	1387	1345	96,97	1 (0,07)
Quadruple Test	539	538	508	94,42	0
Integrated Test	1741	1738	1691	97,30	0

Literature: see www.izotop.hu

CE 1011

WEB site: http://www.izotop.hu
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