

Human Pancreatic & colo-rectal Cancer (CA-242)

For Quantitative Determination of
CA242 In Human Serum

For In Vitro Research Use Only

Human Pancreatic & colo-rectal Cancer (CA-242) ELISA KIT
Cat. No. 0060; Kit Contents: (reagents for 96 tests)

Components	96 tests
Streptavidin coated microwell strip plate (96 wells), Cat. # 0061	1 plate
Human CA-242 Std. A or sample diluent 0 U/mL, 11 ml, Cat. # 062	1 vial
Human CA-242 Std. B, 0.75 ml 5 U/mL, Cat. # 063	1 Vial
Human CA-242 Std. C, 0.75 ml 25 U/mL, Cat. # 064	1 Vial
Human CA-242 Std. D, 50 U/mL 0.75 ml, Cat. # 065	1 Vial
Human CA-242 Std. E, 100 U/mL, 0.75 ml Cat. # 066	1 Vial
Human CA-242 Std. F, 200 U/mL 0.75 ml, Cat. # 067	1 Vial
Biotinylated capture antibody 11 ml Cat. # 068	1 bottle
Anti-CA 242 HRP Conjugate, 11 ml, Cat. # 069	1 bottle
Wash Buffer (20X); 50 ml, Cat# 60-WB	1 bottle
HRP substrate Soln A, Cat. # 060SA	1 bottle
HRP substrate Soln B, Cat. # 060SB	1 bottle
Stop solution, 10 ml, Cat. # T-10	1 bottle
Instruction Manual, M - 0 6 0	1

Insulin is the principal hormone responsible for glucose metabolism. It is synthesized in the cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin and both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain (21 amino acids) and the B chain (30 amino acids), which are linked by two inter-chain disulphide bridges. There is, in addition, a single intra-chain disulphide bridge in the A chain. The sequence of insulin is highly conserved in mammalian species, and is homologous with the insulin-like growth factors IGF-I and IGF-II. Secretion of insulin is mainly controlled by plasma glucose concentration and the hormones have a number of important metabolic actions. Its principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagons, epinephrine (adrenaline), growth hormone and cortisol. Insulin concentrations are severely reduced in insulin-dependent diabetes (IDDM) and some other conditions such as hypopituitarism. Insulin concentrations may be raised in non-insulin-dependent diabetes (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's Syndrome and Acromegaly^{1, 2} The main clinical utility measurement is in the investigation of hypoglycaemia. Insulin assay have been used in the following applications:

1. To assess the residual cell function, especially in newly diagnosed cases of IDDM.
 2. As an aid to the discrimination between IDDM and NIDDM.
 3. The diagnosis of insulinoma.
 4. In the investigation of the pathophysiology of diabetes mellitus.
- Insulin assays are the essentials in various dynamic tests, such as oral of intravenous glucose tolerance tests (OGTT and IVGTT), to determine the insulin response of the pancreas and the degree of insulin resistance. In many applications, insulin measurements may be complicated by cross-reactivity with partially degraded insulin, proinsulin and split forms of proinsulin. Immune complexes of these molecules are essentially problematic in patients who have developed antiinsulin antibodies through animal insulin administration.

PERFORMANCE CHARACTERISTICS

Accuracy:

A serum containing 333 U/ml was diluted with a series of CA242 free serum. The dilutions were tested & the CA242 recoveries were compared with the expected conc.

Intra-assay precision:

Three pooled sera were assayed 8-times in a single run. The inter-assay determinations were performed in duplicate over a period of four days.

Serum samples	Mean (u/mL)	Intra-assay		Mean (u/mL)	Inter-Assay	
		S.D.	CV%		S.D.	CV%
A	54.4	3.05	5.61	53.9	6.29	11.65
B	112.7	6.13	5.43	116.8	8.23	7.06
C	213.5	10.4	4.87	226.4	23.38	10.33

LINEARITY

Samples with known concn were spiked with different conc. Of CA242. Samples were then tested & CA-242 recoveries compared with the expected conc.. As illustrated:

CA242 (u/mL)	CA242 Spiked (u/mL)	Expected (u/mL)	Observed (u/mL)	Recovery (%)
5	25	15	14.3	95.3
5	85	45	45.8	101.8
5	158	81.5	84.7	103.9
25	85	55	56.5	102.7
25	158	91.5	97.3	106.3
100	158	129	117.3	90.9

Sample Dilution	CA242 Expected (u/mL)	CA242 Observed (u/mL)	Recovery (%)
Undiluted	333		
1:1/5	266.5	287	107.7
1:1/4	249.8	244.4	97.8
1:1/3	222	203	91.4
1:1	166.5	165.5	99.3
1:2	111	112.2	101.1
1:4	83	86.3	104.0

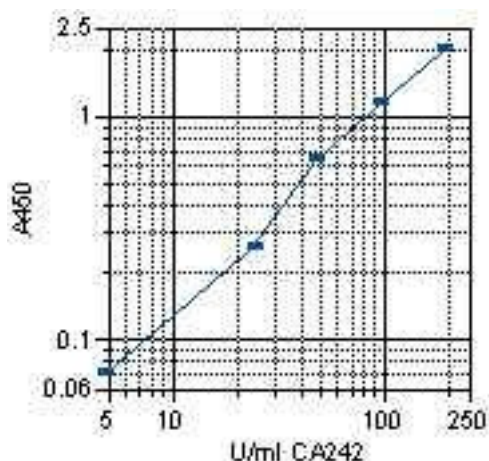
Minimal Detectable Conc.

The detectable limit of CA242 elisa assay is 1 U/mL. The minimal detectable conc. Of CA242 is defined as that of CA 242 which corresponds to the absorbance that is two standard deviation from the mean absorbance of 10 replicate determination of the sample diluent (0/u/mL)

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450nm}	Calculated Conc. (u/mL)
A1, A2	Std. A (0 u/mL)	0.0215	
B1, B2	Std. B (5.0 u/mL)	0.079	
C1, C2	Std. C (25.0 u/mL)	0.277	
D1, D2	Std. D (50 u/mL)	0.683	
E1, E2	Std. E (100 u/mL)	1.226	
F1, F2	Std. F (200 u/mL)	2.084	
G1, G2	Sample 1	1.087	94.5

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Pancreatic & colo-rectal Cancer (CA242) is a solid phase enzyme linked immunosorbent assay (ELISA). This test provides quantitative measurement of CA-242 antigen in human serum. It may be used to research and aid in the clinical evaluation of symptomatic patients suspected of having pancreatic cancer, colo-rectal and other related diseases.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 ul) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Human CA 242 ELISA test is intended for *in vitro* research use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

EXPECTED VALUES

It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its local environmental factors such as diet, climate, etc. A clinical study of the CA-242 quantitative was conducted in the house and results were obtained as follows: Serum samples from 236 normal subjects were assayed and showed that 96 % of the individual have CA-242 values below 15 U/ml and 4% range from 15 to 25 U/ml.

SPECIFICITY

ADI's CA242 ELISA assay only recognizes the CA 242 antigens. The following compounds were tested for cross reactivity of the assay. The cross reactivity to other compounds which might be present in patient samples is not detected at the concentrations given below. Cross reactivity were not found at the concentrations stated with PSA (120 ng/mL), PAP (60 ng/mL), CEA (18248 ng/mL), AFP (10,000 ng/mL), CA-125 (1000/ U/mL). However Crude Antigen CA153 and CA 199 will react with CA 242 in this test. The detectable limit of CA242 ELISA assay is 1 U/mL. The minimal detectable concentration of CA242 is defined as that of CA 242 which corresponds to the absorbance that is two standard deviation from the mean absorbance of 10 replicate determination of the sample diluent (0/mL).

Species Crossreactivity

This kit has been designed and tested for human serum samples. It may be optimized for other biological fluids. It has not been tested in animals (rat, mouse, etc). It will depend upon the crossreactivity of the human antibodies used in the kit with a given animal's hormones/proteins.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate. Do not dilute standards. Dilute Wash buffer 1:20 (50 ml in 950 ml water).
2. Pipet **25 ul of standards**, and available diluted control (not supplied by ADI), and serum samples into appropriate wells in *duplicate*. Immediately dispense 100 ul of biotinylated capture antibody (blue solution). Gently mix the samples, cover the plate and incubate at **room temp for 120 min**.
3. Wash the plate 5X with wash buffer (300 ul/wash). We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing
4. Add **100 ul antibody-enzyme conjugate** into each well. Mix gently. Cover the plate and incubate for **60 minutes** at room temperature
5. Wash the plate 5X with wash buffer (300 ul/wash).
6. Add **200 ul of HRP-substrate mix** at timed intervals into each well (prepared by mixing equal volume of Soln. A and B. Example, for 96 wells, mix 10 mls of Soln A and 10 ml of Soln B in separate clean tube or dispensing tray. Prepare substrate mix as needed. Substrate mix must not be stored). Mix gently, cover the plate and **incubate for 30 min at room temp**. Blue color develops in standard and all positive wells.
7. Stop the reaction by adding **50 ul of stop solution** to all wells. Mix gently. Blue color turns yellow. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES-

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on standard graph paper by plotting net absorbance values of standards against appropriate protein concentrations. Read off the CA-242 concentrations of the control and patient samples.

Testing of other Biological Fluids Species Crossreactivity

This kit is primarily designed to test human serum samples. It is possible to use the plasma and other biological fluids. However, the sample volume or dilutions may be adjusted according to the expected concentrations or unknown samples. Crossreactivity with CA-242 from other species has not been determined.

References: Johansson, C., Nilsson, O., Backstrom, D. : Novel epitopes on the CA50 carrying antigen: Chemical and immunochemical studies. Tumor Biol. 12, 159-179 (1991).