

**ELISA kits available from ADI (see details at the web site)**

- |                     |   |              |                           |
|---------------------|---|--------------|---------------------------|
| <b>#0010</b>        | Human Leptin                                |              |                           |
| <b>#200-120-AGH</b> | Human globular Adiponectin (gAcrp30)        |              |                           |
| <b>#0700</b>        | Human Sex Hormone Binding Glob (SHBG)       |              |                           |
| <b>#0900</b>        | Human IGF-Binding Protein 1 (IGFBP1)        |              |                           |
| <b>#1000</b>        | Human C-Reactive Protein (CRP)              |              |                           |
| <b>#100-110-RSH</b> | Human Resistin /FIZZ3                       |              |                           |
| <b>#100-140-ADH</b> | Human Adiponectin (Acrp30)                  |              |                           |
| <b>#100-160-ANH</b> | Human Angiogenin                            |              |                           |
| <b>#100-180-APH</b> | Human Angiopoietin-2 (Ang-2)                |              |                           |
| <b>#100-190-B7H</b> | Human Bone Morphogenic Protein 7 (BMP-7)    |              |                           |
| <b>#1190</b>        | Human Serum Albumin                         | <b>#1200</b> | Human Albumin (Urinary)   |
| <b>#1750</b>        | Human IgG (total)                           | <b>#1760</b> | Human IgM                 |
| <b>#1800</b>        | Human IgE                                   | <b>#1810</b> | Human Ferritin            |
| <b>#1210</b>        | Human Transferrin (Tf)                      | <b>#0020</b> | Beta-2 microglobulin      |
| <b>#1600</b>        | Human Growth Hormone (GH)                   |              |                           |
|                     |   |              |                           |
| <b>#0060</b>        | Human Pancreatic Colorectal cancer (CA-242) |              |                           |
| <b>#1820</b>        | Human Ovarian Cancer (CA125)                | <b>#1830</b> | Human CA153               |
| <b>#1840</b>        | Human Pancreatic & GI Cancer (CA199)        |              |                           |
| <b>#1310</b>        | Human Pancreatic Lipase                     |              |                           |
| <b>#1400</b>        | Human Prostatic Acid Phosphatase (PAP)      |              |                           |
| <b>#1500</b>        | Human Prostate Specific Antigen (PSA)       | <b>#1510</b> | free PSA (fPSA)           |
| <b>#0500</b>        | Human Alpha Fetoprotein (AFP)               |              |                           |
| <b>#0050</b>        | Human Neuron Specific Enolase (NSE)         |              |                           |
|                     |   |              |                           |
| <b>#0030</b>        | Human Insulin                               | <b>#0040</b> | Human C-peptide           |
| <b>#0100</b>        | Human Luteinizing Hormone (LH)              |              |                           |
| <b>#0200</b>        | Human Follicle Stimulating Hormone (FSH)    |              |                           |
| <b>#0300</b>        | Human Prolactin (PRL)                       |              |                           |
| <b>#0400</b>        | Human Chorionic Gonadotropin (HCG)          | <b>#0410</b> | HCG-free beta             |
|                     |   |              |                           |
| <b>#0600</b>        | Human Thyroid Stimulating Hormone (TSH)     |              |                           |
| <b>#1100</b>        | Human Total Thyroxine (T4)                  | <b>#1110</b> | Human Free T4 (ft4)       |
| <b>#1650</b>        | Human free triiodothyronine (ft3)           | <b>#1700</b> | Human T3 (total)          |
|                     |   |              |                           |
| <b>#1850</b>        | Human Cortisol                              | <b>#1860</b> | Human Progesterone        |
| <b>#1865</b>        | Human Pregnenolone                          | <b>#1875</b> | Human Aldosterone         |
| <b>#1880</b>        | Human Testosterone                          | <b>#1885</b> | Human free Testosterone   |
| <b>#1910</b>        | Human Androstenedione                       | <b>#1920</b> | Human Estradiol           |
| <b>#1925</b>        | Human Estrone                               | <b>#1940</b> | Dihydrotestosterone (DHT) |
| <b>#1950</b>        | Human DHEA-sulphate (DHEA-S)                |              |                           |
| <b>#3400</b>        | Human serum Neopterin                       |              |                           |
|                     |   |              |                           |
| <b>#3000</b>        | Human Rheumatoid Factors IgM (RF)           |              |                           |
| <b>#3100</b>        | Human anti-dsDNA                            |              |                           |
| <b>#3200</b>        | Anti-Nuclear Antibodies (ANA)               |              |                           |

Instruction Manual No. M-0060

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

**ELISA Kit Cat. # 0060, 96 Tests**

For Quantitative Determination of  
CA242 In Human Serum



*For In Vitro Research Use Only*



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## 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), # 0061	1 plate
Human CA-242 <b>Std. A</b> , 0 U/mL, 0.75 ml, # 062A	1 vial
Human CA-242 <b>Std. B</b> , 15 U/mL, 0.75 ml, # 062B	1 Vial
Human CA-242 <b>Std. C</b> , 50 U/mL, 0.75 ml, # 062C	1 Vial
Human CA-242 <b>Std. D</b> , 100 U/mL, 0.75 ml, # 062D	1 Vial
Human CA-242 <b>Std. E</b> , 150 U/mL, 0.75 ml, # 062E	1 Vial
CA-242 control-1, control-2, (exact values printed on vials) 0.75 ml/vial, #CL1-CL2	2 Vials
<b>Biotin Anti-CA242 antibody</b> 15 ml Cat. # 063	1 bottle
Anti-CA 242 <b>HRP Conjugate (20X)</b> , 0.75 ml, # 064	1 vial
<b>Conjugate Diluent</b> , 15 ml, # 065	1 bottle
<b>Wash Buffer (25X)</b> ; 50 ml, # 60-WB	1 bottle
HRP substrate Solution, 12 ml # TMB-60	1 bottle
Stop solution, 15 ml, # T-10	1 bottle
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### Intended Use:

ADI's CA-242 ELISA kit is intended for the quantitative determination of CA242 cancer antigen in Human serum. For In Vitro Research Use Only (RUO).

### Introduction:

The CA-242 is sialylated carbohydrate antigen present on mucinous type of glycoproteins in carcinomas of many organs. The CA-242 antigen is released from the tumor and the CA-242 can be detected in serum from patients with carcinomas. In the normal healthy subjects and subjects with benign diseases, the CA-242 levels are low, while elevated levels are commonly found in patients with gastro-intestinal cancer. By identifying the colo-rectal cancer patients at an early stage of the disease, primary diagnosis, often relies on occult blood testing, and on radiological endoscopic examination of the large bowel. CEA is widely used for the monitoring and prognostic assessment of patients with colo-rectal cancer while the clinical utility of CEA is limited due to the low sensitivity in early stages of cancer. CEA showed higher sensitivity for rectal cancer than for colonic cancer, while the opposite was true for CA-242. However, a combination of CA-242 with CEA will improve with higher sensitivity for both rectal and colonic cancer. CA-242 is better than CA-199 in the diagnosis of pancreatic cancer because of its higher specificity, and it may be useful in the screening of localized or respectable tumors.

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.

## 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.

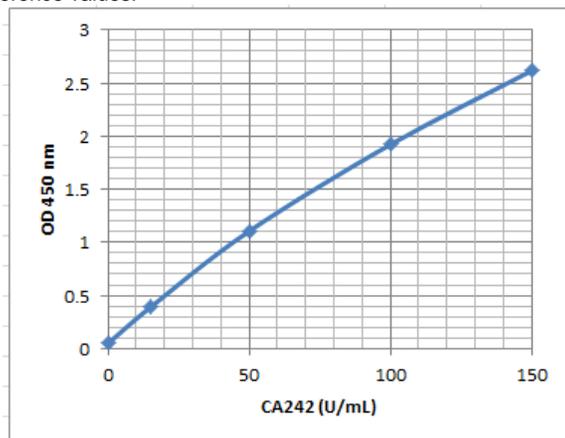
The detectable limit of CA242 elisa assay is 1 U/mL.

**References:** Johansson, C Tumor boil.12, 159-179 (1991); Johansson, C. CanAg. Int J. Cancer 48, 757-763 (1991); Nilsson, O., Br. J Cancer 65, 215-221 (1992); Kawa, Br. J. Cancer, 70,

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A <sub>450nm</sub>	Calculated Conc. (u/mL)
A1, A2	<b>Std. A</b> (0 u/mL)	0.050	
B1, B2	<b>Std. B</b> (15 u/mL)	0.390	
C1, C2	<b>Std. C</b> (50 u/mL)	1.107	
D1, D2	<b>Std. D</b> (100 u/mL)	1.922	
E1, E2	<b>Std. E</b> (150 u/mL)	2.617	
F1, F2	<b>Sample 1</b>	0.410	16.1

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.



Kit-spec-XL

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

### 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 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. If a microplate spectrophotometer reader with built-in data calculation program is used, refer to the manual for the plate reader and create a program using the concentration stated on the labels of each of the CA242 Calibrators.

For automatic calculation of CA242 results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0 U/mL.
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0 U/mL.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 U/mL.

**NOTE:** 4-parametric or linear regression should not be used.

## PRINCIPLE OF THE TEST

Pancreatic & rectal CA-242 cancer quantitative Assay is a solid phase enzyme-linked immunosorbent system employing plastic wells coated with streptavidin. The sample, standards and controls and biotinylated anti-CA- 242 antibodies are allowed to incubate in the wells. During the incubation, specific cancer antigen(CA-242) is bound to CA-242 antibodies on the wells. Unbound CA-242 antigen is removed by washing the wells with buffer. Enzyme conjugate is added to each well. After the incubation, unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the CA -242 antigen present in the sample. Upon addition of the chromogen substrate, the intensity of color developed is proportional to the concentration of CA-242 antigen in the sample and may be quantified by use of a photometric well reader at 450 nm wavelength.

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 Alpha Diagnostic Intl., Inc. 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.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H<sub>2</sub>SO<sub>4</sub> (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

[http://4adi.com/commerce/info/showpage.jsp?page\\_id=1060&category\\_id=2430&visit=10](http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10)

### 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.

### Reagent Preparation

**Dilute wash buffer (1:25) with distilled water (50 ml stock in 1200 ml).** Store at 4oC.

**Prepare 1X Anti-CA242- HRP conjugate stock (20X).** Dilute 50 ul stock conjugate per ml of conjugate diluent. Prepare 10-ml for a full plate (500 ul in 10 ml for complete 96-well plate). Prepare in required volumes and do not store working conjugate beyond the assay date.

## 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 or date printed. 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. Reagents should be allowed to reach room temp. (20-28 °C).

1. Label or mark the microtiter well strips to be used on the plate. Do not dilute standards. **Dilute Wash buffer 1:25** (50 ml in 1200 ml water) and **Dilute HRP conjugate stock 1:20** with conjugate diluent (**50 ul** of conjugate stock into **1 ml of conjugate diluent**).
2. Pipet **25 ul of standards**, and available control, and serum samples into appropriate wells in *duplicate*. Immediately dispense **100 ul of biotin Anti-CA242 antibody**. Gently mix the samples, cover the plate and incubate at **room temp (25-28oC) for 120 min** on an orbital shaker100-150 rpm).
3. Wash the plate **3X 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 diluted HRP conjugate** into each well. Mix gently. Cover the plate and incubate for **60 minutes** at room temperature on an orbital shaker100-150 rpm).
5. Wash the plate **6X with wash buffer** (300 ul/wash).
6. Add **100 ul of HRP-substrate** at timed intervals into each well. Mix gently, cover the plate and **incubate for 30 min at room temp**. Blue color develops into standard and all positive wells. It is possible to read the plate at 620nm but the color will continue to increase with time.
7. Stop the reaction by adding **100 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.

## 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.

## 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