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

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1840

Pancreatic & GI Cancer (Mucin PC/CA199)

ELISA KIT Cat. No. 1840

For Quantitative Determination of CA199 In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Pancreatic & GI Cancer CA199/CA19-9 ELISA KIT Cat. No. 1840
For Quantitative Determination of CA199 In Serum
Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	
CA19-9 antibody coated microwell strip plate (96 wells), Ready-to-use #1 8 4 1	1 P l a t e
CA199 **Standard A** (0 U/ml), 0.5 ml, #1 8 4 2 A	1 V i a l
CA199 **Standard B** (25 U/ml), 0.5 ml, #1 8 4 2 B	1 V i a l
CA199 **Standard C** (75 U/ml), 0.5 ml , #1 8 4 2 C	1 V i a l
CA199 **Standard D** (150 U/ml), 0.5 ml, #1 8 4 2 D	1 V i a l
CA199 **Standard E** (300 U/ml), 0.5 ml, #1 8 4 2 E	1 V i a l
CA199 **Standard F** (600 U/ml), 0.5 ml, #1 8 4 2 F	1 V i a l
Assay Buffer, 13 ml , #1843	1 b o t t l e
Anti-CA19-9-HRP **Conjugate conc.** (12X); 1.1 ml , #1844	1 b o t t l e
Conjugate Diluent, 13 ml, #1845	1 b o t t l e
HRP substrate Solution; 11 ml, #TMB-1840	1 b o t t l e
Wash buffer (20X), 50 ml (2X25 ml) **(**dilute 1:20 with distilled water), #WB-1840	2 b o t t l e s
Stop solution, 11 ml, #ST-1840	1 b o t t l e
Complete Instruction Manual, M 1 8 4 0	1

Intended use:

ADI's CA199 ELISA kit provides for the measurement of CA199 in Human serum for monitoring patients with pancreatic & GI cancer. For in vitro research use only (RUO).

Introduction

The assay for Pancreatic and Gastro-Intestinal Cancer marker (PC-199) measures a carbohydrate antigenic determinant expressed on a high molecular weight mucin. This mucin type of glycoprotein is found in the area of pancreatic and colon and hepatocellular carcinomas. PC 19-9 (CA 199) is also related to the Lewis blood group substances and only serum antigen from cancer patients belonging to the Le (a+b-) or Le (a-b+) blood group will be CA 19-9 positive.

The Pancreatic & Gastro-Intestinal (PC) assay may have two clinical applications:

1. To identify patients having gastric and pancreatic carcinomas.
2. To monitor therapy and tumor recurrence

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standards, the minimum concentration of human CA199 detected using this assay is 10 U/ml. The detection limit is defined as the value deviating by 3 SD from the zero standards.

2. PRECISION

Intra-assay precision:

Two serum samples were run in ten replicates in an assay. The samples showed good intra-assay precision (5-10%CV). The actual values were: mean 22.9 U/ml, SD 4.2 U/ml and 57.2 U/ml, SD 3.70 U/ml.

Inter-assay precision:

Two serum samples were run in duplicate in 7 independent assays. The samples showed good inter-assay precision (6-9 %CV). The actual values were: mean 20.8 U/ml, SD 9.4 U/ml, %CV 9.4; mean 60.5 U/ml, SD 4.00 U/ml, %CV 6.60.

3. RECOVERY

A known amount of CA199 (150 U/ml) was added to 4 samples with initial CA199 of 5-200 U/ml and the total CA199 concentrations measured. The assay showed excellent mean recoveries of about 85-101%).

General References: Steinberg W et al (1990) Am. J. Gastroenterology 4, 350-355; DelVillano BC et al (1983) Clin. Chem.29, 549-552; Glenn J et al (1988) J. Clin. Pathol. 3, 462-468

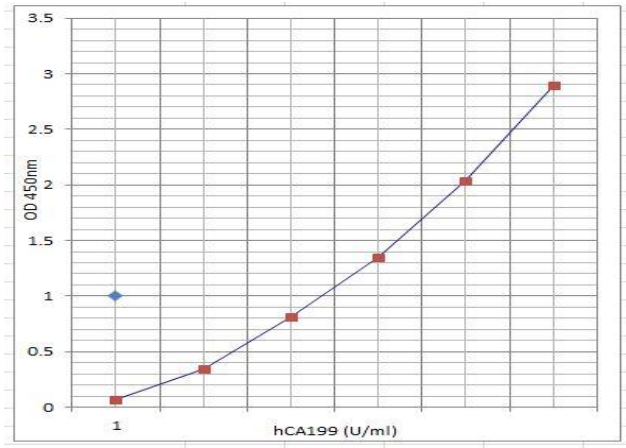
(2) Citations of ADI's ELISA kits (see web site for updated list)

Koopman J, 2004, Clin. Cancer Res., 10: 860 – 868, CA199 elisa serum
Koopman J, 2004, Clin. Cancer Res., 10: 2386 – 2392, CA199 elisa serum

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (U/ml)	Net Mean $A_{450\ nm}$
A1, A2	**Std. A** (0)	0.065
B1, B2	**Std. B** (25 U/ml)	0.342
C1, C2	**Std. C** (75 U/ml)	0.811
D1, D2	**Std. D** (150 U/ml)	1.349
E1, E2	**Std. E** (300 U/ml)	2.031
F1, F2	**Std. F** (600 U/ml)	2.898

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.



CA19-9 (U/ml)

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 standards from the mean absorbance values of standards, control, and samples. Draw the standard curve on linear graph paper by plotting net absorbance values of standards against appropriate CA199 concentrations. Read off the CA199 concentrations of the control and patient samples. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.

PRINCIPLE OF THE TEST

CA199 ELISA kit is a solid phase ELISA. The wells are coated with Streptavidin. The samples, std., and controls, and biotinylated anti-PC antibody are allowed to bind to Streptavidin-coated plates. During the incubation, CA199 antigen is bound to anti-PC antigen antibodies on the wells. Unbound PC antigen is removed by washing the wells with buffer. Enzyme conjugate is then added to all wells. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (blue color) is directly proportional to the amount of CA199 present in the sample. Adding stopping solution terminates the reaction and converts blue color into yellow. Absorbance is then measured on an ELISA reader at 450 nm. and the concentration of CA199 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

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

TMB (substrate), Hcl (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

PRECAUTIONS

The Alpha Diagnostic International CA199 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^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Dilute wash buffer (1:20) with distilled water (50 ml stock in total of 1-liter**).**
Store at 4oC.

Dilute **HRP Enzyme conjugate conc**.1:11; with **conjugate diluent** (add 1 ml stock in 11 ml).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8oC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Reconstituted control serum is stable for one week at 2-8oC. The unused portions of the standards should 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.

1. **Dilute wash buffer (1:20) with distilled water (50 ml stock in 950 ml).** Label or mark the microtiter well strips to be used on the plate.

2. Pipet **10 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.

3. Add **100 ul of Assay buffer** (green color) into each well and incubate for **90 min** at **37oC**.

4. Remove incubation mixture and **wash the wells 4X** with wash buffer. 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.

5. Add **100 ul of 1X anti-CA199-HRP** conjugate into **each well.** Mix gently. Cover the plate and incubate for **90 minutes** at **37oC**..

6. Remove reaction mixture and **wash 4X** with wash buffer.

7. Dispense **100 ul TMB substrate per well.** Mix the plate gently for 5-10 seconds. Cover the plate and incubate at **room temp** (25-28oC). for **20 minutes.** Blue color develops in standards and positive wells.

8. Stop the reaction by adding **100 ul of stop solution** to **all wells.** Mix gently for 5-10 seconds (blue color turns yellow). Measure the **A450 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^{o}C.

Addition of the HRP substrate solution starts a kinetic reaction. 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.

DILUTION OF SAMPLES

Serum samples containing more than 600 U/ml of CA199 should be diluted with the zero standard (standard A), reassayed, and the results obtained should be multiplied by the appropriate dilution factor.

EXPECTED VALUES

It is recommended that each laboratory must determine its own normal and abnormal ranges. It is reported that PC tumor marker assay (benign conditions) results between 0-37 U/ml are considered normal. The range between 37-70 U/ml is considered to be a “gray zone” because patients with chronic pancreatitis and pancreatic cancer may exhibit levels ion this range. Assay results >70 U/ml represent a high probability of malignancy. The PC assay is also a prognostic indicator as an adjunct to chemotherapy (1).

LIMITATIONS

ADI's CA199 ELISA kit should be used in conjunction with other data available to the physicians. This kit is designed to avoid high dose hook effect.

Samples with PC antigen levels above 260 U/ml should be diluted to obtain an accurate value.

Species Reactivity

This kit is designed to detect human CA199/CA 19-9 and it has not been tested in species such as mouse, rat, monkey etc. Human tumor or cells transplanted into animals (mouse or rat) that secrete human CA19-9 may be detected with the human kit provided the antigen concentrations are within the detectable range of the kit.