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 Folicle 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 (toal)

#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-1830

Mucin Breast Cancer (CA153)

ELISA KIT Cat. No. 1830

For Quantitative Determination of CA153 In Human Serum

For In Vitro Research Use Only

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

Mucin Breast Cancer ELISA KIT Cat. No. 1830

For Quantitative Determination of CA153 In Serum
Kit Contents: (reagents for 96 tests)

Components	Cat. #
Streptavidin coated microwell **strip plate** (96 wells), Ready-to-use	1831
CA153 **Standard A** (0 U/ml), 11 ml	1832
CA153 **Standard B** (5 U/ml), 0.50 ml	1833
CA153 **Standard C** (25 U/ml), 0. 50 ml	1834
CA153 **Standard D** (50 U/ml), 0. 50 ml	1835
CA153 **Standard E** (100 U/ml), 0. 50 ml	1836
CA153 **Standard F** (200 U/ml), 0. 50 ml	1837
Anti-CA153-HRP **Conjugate**; 11 ml	1838
Biotinylated Capture **Antibody Soln**; 11 ml	1839
HRP substrate **Solution A**; 11 ml	1830SA
HRP substrate **Solution B**; 11 ml	1830SB
Wash Buffer (20X) 50 ml	W20
Stop solution, 10 ml	T-10
Complete Instruction **Manual**	M1830

Introduction

The MBC antigen is a membrane anchored mucin type glycoprotein present in a variety of adenocarcinomas including breast, colon, ovary, lung and pancreas, and normal epithelial cells of different organs. The mucin (MBC) is secreted from tumor cells and can be used as serological marker of breast cancer. Several commercial breast cancer assays measuring the MBC breast antigen are available under different brand name, e.g. CA 15-3. Before the introduction of CA 15-3, carcinoembroynic antigen (CEA) was commonly used to monitor breast cancer patients. The CA 15-3 or mucin breast cancer assay is a more sensitive and specific marker in breast cancer than CEA.

Mucin breast cancer marker correlates with disease progression, regression, or stability in higher number of patients than CEA. The mucin breast cancer assay may have two clinical applications: (i) to identify patients most likely to develop metastatic disease and (ii) to monitor therapy and tumor recurrence.

ADI's CA153 ELISA kit provides for the measurement of CA153 in serum for monitoring patients with breast cancer.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of human CA153 detected using this assay is 3 U/ml. The detection limit is defined as the value deviating by 3 SD from the zero standard.

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 15.16 U/ml, SD 1.70 and 27.89 U/ml, SD 2.64.

Inter-assay precision:

Two serum samples were run in duplicate in eight independent assays. The samples showed good inter-assay precision (8-10 %CV). The actual values were: mean 15.16 U/ml, SD 0.08 U/ml, %CV 8.0; mean 30.2 U/ml, SD 0.17 U/ml, %CV 12.28.

3. RECOVERY

A known amount of CA153 (10-30 U/ml) was added to three samples with initial CA153 of 9.5 U/ml and the total CA153 concentrations measured. The assay showed excellent mean recoveries of about 88-94%).

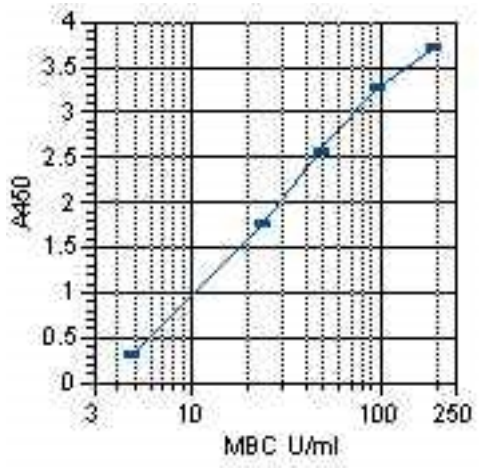
References

1. Jacob et al (1990) in RB Heberman Eds. P 69-82
2. Burchell J et al (1989) Intl J. Cancer 44, 691-696.
3. Ceraini RI et al (1982) PNAS 79, 5420-5424
4. Hayes DF 382) PNAS 79, 5420-5424
5. Hayes DF et al (1986) J Clin Oncol. 4, 1542-1550.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (U/ml)	Net Mean $A_{450\ nm}$	Calculated Conc. (U/ml)
A1, A2	**Std. A** (0)	0.075	
B1, B2	**Std. B** (5 U/ml)	0.340	
C1, C2	**Std. C** (25 U/ml)	1.050	
D1, D2	**Std. D** (50 U/ml)	1.700	
E1, E2	**Std. E** (100 U/ml)	2.050	
F1, F2	**Std. F** (200 U/ml)	2.250	
G1, G2	**Sample 1**	0.810	16.60

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

CA153 ELISA kit is a solid phase ELISA. The wells are coated with Streptavidin. The samples, std., and controls, and biotinylated anti-CA153 antibody are allowed to bind to Streptavidin-coated plates. During the incubation, CA153 antigen is bound to anti- CA153 antigen antibodies on the wells. Unbound CA153 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 CA153 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 CA153 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.

PRECAUTIONS

The Alpha Diagnostic International CA153 ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 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), HCl (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate 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

Before use, dilute wash buffer (1:20) with distilled water.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C 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.

HRP substrate buffer (solution A) and HRP substrate (solution B) should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. Reconstituted control serum is stable for one week at 2-8^{o}C. 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).*

1. Dilute wash buffer (1:20) with distilled water. Label or mark the microtiter well strips to be used on the plate. Dispense 200-300 ul of wash buffer or water 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.
2. Pipet 10 ul of standards, control, and serum samples into appropriate wells in *duplicate*.
3. Add 100 ul of biotinylated capture antibody into each well. Mix gently for 5-10 seconds and incubate for **60 min** at room temp.
4. Remove incubation mixture and wash the wells 5X with wash buffer.
5. Add 100 ul of anti-CA153-HRP conjugate into **each well.** Mix gently. Cover the plate and incubate for **60 minutes** at room temperature. 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.
6. Remove reaction mixture and wash 5X with wash buffer.
7. Premix HRP substrate Solution A and B (1:1 v/v) and dispense 200 ul per well (need 20 ml for full plate). Do not keep mixed solution for more than 10-20 min and prepare only in required amounts. Make sure that TMB solution is at room temp before mixing and dispensing into the plate. Mix the plate gently for 5-10 seconds. Cover the plate and incubate at room temp. for **30 minutes.** Blue color develops in standards and positive wells
8. Stop the reaction by adding **50 ul of stop** solution to all wells**.** Mix gently for 5-10 seconds (blue color turns yellow).
9. 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^{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 200 U/ml of CA153 should be diluted with the zero standard (standard A), reassayed, and the results obtained should be multiplied by the appropriate dilution factor.

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 log-log graph paper by plotting net absorbance values of standards against appropriate CA153 concentrations. Read off the CA153 concentrations of the control and patient samples.

EXPECTED VALUES

It is recommended that each laboratory must determine its own normal and abnormal ranges. It is reported that <2% of normal healthy individuals have CA153 >30 U/ml.

LIMITATIONS

ADI's CA153 ELISA kit should be used in conjunction with other data available to the physicians. This kit is designed to avoid high dose hook effect.