Version 30-04/24

User's Manual

CEA ELISA Kit

REF **DEIA2284**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Immunoradiometric assay kit for the in vitro quantitative measurement of human Carcino Embryonic Antigen (CEA) in serum.

General Description

1. Carcino Embryonic Antigen (CEA)

CEA is a 200.000 Daltons oncofetal glycoprotein expressed by normal tissues during the first six months of fetal life. Later on the expression of CEA by normal cells becomes largely repressed except in cancer tissues of various cell types, which may secrete large amounts of this oncofetal protein into the circulation. Widely accepted as a useful adjunct for monitoring the course of cancer diseases, CEA should not be regarded as a tumor-specific marker because it is still secreted in small amounts by certain normal tissues during adult life, with small serum level increases in case of benign diseases such as cirrhosis, hepatitis, inflammatory bowel diseases, renal failure and in heavy smokers. Therefore, the measurement of CEA serum concentration for diagnostic purposes must be considered with great care.

2. Clinical applications

Monitoring of cancer diseases

When measured before any therapy, the serum concentration of CEA is one of the best parameters to monitor the evolution of cancer following surgery, chemotherapy, etc. After remission, CEA levels appear often as a good screening test for early detection of tumor recurrence.

Diagnostic adjunct in cancer

Although not specific for cancer when elevated to less than 20 ng/ml, CEA levels above this limit are highly suggestive of malignancy (less than 0.5% false positive).

Prognostic adjunct in cancer

CEA levels in serum provide important prognostic information because a direct relationship has been established between CEA serum concentration and Dukes classification in colon. The same relationship exists probably also in mammary carcinoma and lung carcinoma where very high CEA levels occur almost exclusively in case of disseminated metastasis.

Principles of Testing

The CEA-IRMA is an immunoradiometric assay based on coated-tube separation. Mabs1, the capture antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labelled with ^{125}I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyperspecificity, common to two-site IRMA, as well as a need of a shaker or long incubation at 37°C.

Reagents And Materials Provided

Reagents	96 tests Kit	Colour Code	Reconstitution
Tubes coated with anti CEA (monoclonal antibodies)	2 x 48	violet	Ready for use
Ab ^{125}I: Anti-CEA-^{125}I (monoclonal antibodies) in phosphate buffer with bovine serum albumin, azide (<0.1%), EDTA and inert red dye	1 vial 5.5 ml 440 kBq	red	Ready for use
DIL SPE: Specimen diluent in human serum with thymol	1 vial lyophil.	black	Add distilled water (see exact amount on vial label)
CAL N: Calibrators 0-5 in human serum with thymol (see exact value on vial labels)	6 vials lyophil.	yellow	Add 1.0 ml distilled water
WASH SOLN CONC: Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70× with distilled water (use a magnetic stirrer).
CONTROL N: Controls 1 and 2 in human serum and thymol	2 vials lyophil.	silver	Add 0.5 ml distilled water

Note:

1. Use the content of the diluent vials for sera dilutions.
2. 1 IU of the calibrator is equivalent to 1 IU of the 1st IRP of human CEA 73/601. 1 IU of the calibrator is equivalent to 100 ng.

Materials Required But Not Supplied

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 500 µl and 1000 µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Pipette for delivery of 5 to 10 ml of distilled water
4. Vortex mixer
5. Magnetic stirrer
6. Incubator at 37°C
7. 5 ml automatic syringe (Cornwall type) for washing
8. Aspiration system (optional)
9. Any gamma counter capable of measuring 125I may be used (minimal yield 70%).

Storage

1. Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
2. After reconstitution of the calibrators and controls, aliquots should be made and kept at –20°C for maximum 3 months.
3. Reconstituted specimen diluent is stable for 8 weeks at 2 to 8°C.

4. Freshly prepared Working Wash solution should be used on the same day.
5. After its first use, tracer is stable until expiry date, if kept in the original well closed vial at 2 to 8°C.
6. Alterations in physical appearance of kit reagents may indicate instability or deterioration.

Specimen Collection And Preparation

1. Serum must be kept at 2-8°C.
2. If the test is not run within 24 h, storage at -20°C is recommended.
3. Avoid subsequent freeze-thaw cycles.
4. Do not use plasma samples.

Reagent Preparation

1. Calibrators: Reconstitute the calibrators 0-5 with 1.0 ml distilled water.

2. Controls: Reconstitute the controls with 0.5 ml distilled water.

3. Working Wash solution: Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70×). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

4. Diluent: Reconstitute the diluent with the amount of distilled water as mentioned on the vial label.

Assay Procedure

1. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

2. Procedure

a. Label coated tubes in duplicate for each calibrator, sample and control. For determination of total counts, label 2 normal tubes.

b. Briefly vortex calibrators, samples, controls and dispense 100 µl of each into the respective tubes.

c. Dispense 50 µl of anti-CEA-^{125}I tracer into each tube, including the uncoated tubes for total counts.

d. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.

e. Incubate for 2 hours at 37°C.

f. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.

g. Wash tubes with 2 ml Working Wash solution (except total counts). Avoid foaming during the addition of the Working Wash solution.

h. Aspirate (or decant) the content of each tube (except total counts).

i. Wash tubes again with 2 ml Wash solution (except total counts) and aspirate (or decant).

j. After the last washing, let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.

k. Count tubes in a gamma counter for 60 seconds.

Quality Control

1. If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
2. If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Do not freeze-thaw more than twice.
3. Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

Calculation

1. Calculate the mean of duplicate determinations.
2. On semi logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of CEA (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is to be used, a 4-parameter logistic function curve fitting is recommended.

Typical Standard Curve

The following data are for illustration only and should never be used instead of the real time calibration curve.

CEA-IRMA		cpm	B/T (%)
Total count		216032	100
Calibrator	0.0 ng/ml	679	
	2.0 ng/ml	1581	0.42
	6.0 ng/ml	3621	1.36
	20.0 ng/ml	8620	3.68
	60.0 ng/ml	23790	10.70
	200.0 ng/ml	64556	29.57

Reference Values

Reference intervals

These values are given only for guidance; each laboratory should establish its own normal range of values.

% DISTRIBUTION OF CEA VALUES					
	Number	**0-3.0 ng/ml**	**3.1-5.0 ng/ml**	**5.1-10.0 ng/ml**	**> 10 ng/ml**
Healthy					
Non-smokers	110	96.4	2.7	0.9	0
Smokers	64	78.1	10.9	7.8	3.1
TOTAL	174	89.7	5.7	3.4	1.1
Non-malignant					
Cirrhosis	37	29.7	13.5	37.8	18.9
Crohn	26	88.5	7.7	0	3.8
Malignant					
Colorectal	58	31.0	6.9	10.3	51.7
Mammary	50	60.0	14.0	10.0	16.0
Gastric	61	42.6	8.2	13.1	36.1
Pulmonary	50	30.0	12.0	22.0	36.0
Ovarian	50	84.0	2.0	8.0	6.0

Performance Characteristics

Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to the coated tubes.

TIME DELAY				
	0'	10'	20'	30'
S 1 (ng/ml)	7.8	8.1	8.0	8.0
S 2 (ng/ml)	27.0	26.4	26.7	25.8

Hook-effect

A Hook effect can be observed above 25000 ng/ml CEA.

Precision

INTRA ASSAY				INTER ASSAY			
Serum	**Replicate**	**<X> ± SD (ng/ml)**	**CV (%)**	**Serum**	**Replicate**	**<X> ± SD (ng/ml)**	**CV (%)**
A B	20 20	12.2 ± 0.3 22.8 ± 0.8	2.3 3.1	A B	20 20	5.3 ± 0.4 21.4 ± 1.1	7.4 5.1

SD : Standard Deviation; CV: Coefficient of variation

Detection Limit

Twelve zero calibrators were assayed along with a set of the other calibrators. The detection limit, defined as the apparent concentration of the average count at zero binding plus two standard deviations, was 0.17 ng/ml

Specificity

Cross reactivity with the normal cross-reacting antigens NCA & NCA-2 and mal-CEA & mbp-CEA was evaluated. Serum samples were spiked with various amounts of NCA, NCA-2, mal-CEA or mbp-CEA as shown in the table below.

Cross reactivity with NCA is a known phenomenon that is also observed with other CEA assays.

sample	composition	Theoretical concentration (ng/ml)	CEA-IRMA result (ng/ml)
C051	-	-	2.3
C052	C051 + NCA-2 (82 ng/ml)	2.3	5.7
C053	C051 + NCA-2 (163 ng/ml)	2.3	6.6
C066	-	-	5.9
C076	C066 + CEA (73/603 at 100 U/L)	15.9	15.8
C068	C066 + CEA (73/603 at 100 U/L) + NCA-2 (82 ng/ml)	15.9	18.5
C069	C066 + NCA-2 (82 ng/ml)	5.9	6.9
C081	-	-	3.7
C082	C081 + 1 µg/ml Mal CEA	3.7	3.5
C087	+ CEA (73/603 at 100 U/L)	-	14
C088	C087 + 8 µg/ml mbp-CEA	14	14.3
C089	C087 + 2 µg/ml NCA	14	49.6

Linearity

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
1	1/1	195.2	195.2
	1/2	97.6	100.1
	1/4	48.8	57.2
	1/8	24.4	30.2
	1/16	12.2	13.6
	1/32	6.1	7.0
	1/64	3.1	3.4
	1/128	1.5	2.8
2	1/1	164.8	164.8
	1/2	82.4	89.7
	1/4	41.2	52.2
	1/8	20.6	25.6
	1/16	10.3	11.0
	1/32	5.2	6.4
	1/64	2.6	3.5
	1/128	1.3	1.1

Samples were diluted with specimen diluent.

Recovery

RECOVERY TEST

Added CEA (ng/ml)	Recovered CEA (ng/ml)	Recovery (%)
5	4.7	94.0
12.5	11.1	88.8
35	30.9	88.3
50	45.5	91.0
100	98.4	98.4

Precautions

Safety

For research use only. This kit contains ^{125}I (half-life: 60 days),emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the

product must be administered to humans or animals.

All radioactive handling should be executed in a designated area. away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections.

Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up. Do not smoke, drink, eat or apply cosmetics in the working area.

Do not pipette by mouth. Use protective clothing and disposable gloves.

Limitations

1. Specimens from patients who have received preparations of mouse monoclonal antibodies for research may contain human antimouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.