



# CanAg CEA EIA

**For Research Use Only.  
Not for use in  
diagnostic procedures.**

**REF 401-85**

Instructions for use. 2006-10

Enzyme immunometric assay kit  
For 96 determinations

**GB EXPLANATION OF SYMBOLS**  
**DE BEDEUTUNG DER SYMBOLE**  
**ES EXPLICACIÓN DE SÍMBOLOS**  
**IT SIGNIFICATO DEI SIMBOLI**  
**FR EXPLICATION DES SYMBOLES**  
**DK SYMBOLFORKLARING**  
**GR ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ**  
**SE SYMBOLFÖRKLARING**



Use By/Verwendbar bis/  
Fecha de caducidad/  
Utilizzare entro/Utiliser jusque/  
Holdbar til/Ημερομηνία λήξης/  
Bäst före datum

**LOT**

Batch code/Chargenbezeichnung/  
Codigo de lote/  
Codice del lotto/Code du lot/  
Lotnummer/Αριθμός Παρτίδας/  
Lotnummer



Date of manufacture/  
Herstellungsdatum/  
Fecha de fabricación/  
Data di fabbricazione/  
Date de fabrication/  
Produktionsdato/  
Ημερομηνία Παραγωγής/  
Tilverkningsdatum

**REF**

Catalogue number/Bestellnummer/  
Número de catálogo/  
Numero di catalogo/  
Référence du catalogue/  
Katalognummer/  
Αριθμός καταλόγου/  
Produktnummer



Manufacturer/Hersteller/Fab-  
ricante/Fabbricante/Fabricant/  
Προδουcent/τασκευαστής/  
Tilverkare



Contains sufficient for <96> tests/  
Ausreichend für "96" Ansätze/  
Contenido suficiente  
para <96> ensayos/  
Contenuto sufficiente per "96" saggi/  
Contenu suffisant pour "96" tests/  
Indeholder tilstrækkeligt  
til "96" test/  
Περιεχόμενο επαρκές  
για «96» εξετάσεις/  
Innehåller tillräckligt till "96" tester



Temperature limitation/  
Zulässiger Temperaturbereich/  
Limite de temperatura/  
Limiti di temperatura/  
Limites de température/  
Temperaturbegrænsning/  
Περιορισμοί θερμοκρασίας/  
Temperaturgräns



Consult Instructions for Use/  
Gebrauchsanweisung beachten/  
Consulte las instrucciones de uso/  
Consultare le istruzioni per l'uso/  
Consulter les instructions d'utilisation/  
Se brugsanvisning/  
Συμβουλευτείτε τις οδηγίες  
χρήσης/  
Se bruksanvisning



Biological risks/Biogefährdung/  
Riesgo biológico/Rischio biologico/  
Risques biologiques/  
Biologisk fare/Βιολογικοί κίνδυνοι/  
Biologisk risk



Contents of kit/Inhalt/Contenido/  
Contenido/Contenu/Inndhold/  
ανιδραστήρια/Kit innehåll



From mouse/der Maus/de ratón/  
Murino/De souris/Mus/απο ποντίκι/  
Från mus



Human/Human/Humano/  
Origine Umana/Humaine/Human  
δείγματα αναφοράς/Human

## INTENDED USE

The CanAg CEA EIA kit is intended for the quantitative determination of the cancer associated antigen CEA in serum.

## SUMMARY AND EXPLANATION OF THE ASSAY

Carcinoembryonic antigen (CEA) is a glycoprotein, which was first identified in patients with colonic carcinoma and in epithelial tumours of endodermal origin (gastrointestinal tract) by Gold and Freedman (1). The CEA molecule is quite heterogeneous due to the carbohydrate contents (50-60%) and depending on the purification procedure employed. It is soluble in perchloric acid and has a molecular weight of about 175.000–200.000 Daltons (2). Immunological and genetic characterization of CEA has identified a family of CEA-like molecules sharing common antigenic determinants. The most relevant CEA-like molecule is NCA (non-specific cross-reacting antigen) synthesized both by normal and pathological tissues. The problem of cross-reacting CEA-like molecules when assaying CEA is possible to overcome by the use of monoclonal antibodies. The CanAg CEA EIA is based on two mouse monoclonal antibodies against the Gold epitopes IV and V (3, 4).

## PRINCIPLE OF THE TEST

The CanAg CEA EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators, controls and samples are incubated together with biotinylated Anti-CEA monoclonal antibody and horseradish peroxidase (HRP) labelled Anti-CEA monoclonal antibody in Streptavidin coated microstrips. After washing, buffered Substrate/ Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour is proportional to the amount of CEA present in the samples.

The colour intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution). Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The CEA concentrations of samples are then read from the calibration curve.

## REAGENTS

- Each CanAg CEA EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8°C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

Component	Quantity	Storage and stability after first opening
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### MICROPLA

Microplate	1 Plate	2–8°C until expiry date stated on the plate
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12 x 8 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

Component	Quantity	Storage and stability after first opening			
<b>CEA Calibrators</b>	6 vials	2–8°C until expiry date stated on the vials			
<table border="1"><tr><td>CAL</td><td>CEA</td><td>0</td></tr></table>	CAL	CEA	0	0 µg/L 1 x 8 mL	
CAL	CEA	0			
<table border="1"><tr><td>CAL</td><td>CEA</td><td>2</td></tr></table>	CAL	CEA	2	2 µg/L 1 x 0.75 mL	
CAL	CEA	2			
<table border="1"><tr><td>CAL</td><td>CEA</td><td>5</td></tr></table>	CAL	CEA	5	5 µg/L 1 x 0.75 mL	
CAL	CEA	5			
<table border="1"><tr><td>CAL</td><td>CEA</td><td>15</td></tr></table>	CAL	CEA	15	15 µg/L 1 x 0.75 mL	
CAL	CEA	15			
<table border="1"><tr><td>CAL</td><td>CEA</td><td>50</td></tr></table>	CAL	CEA	50	50 µg/L 1 x 0.75 mL	
CAL	CEA	50			
<table border="1"><tr><td>CAL</td><td>CEA</td><td>75</td></tr></table>	CAL	CEA	75	75 µg/L 1 x 0.75 mL	
CAL	CEA	75			

Human CEA in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye and 0.01% methyl-isothiazolone (MIT) as preservative. Ready for use. 

CAL	CEA	0
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 should also be used for dilution of samples.

<b>CEA Controls</b>	2 vials	2–8°C until expiry date stated on the vials			
<table border="1"><tr><td>CONTROL</td><td>CEA</td><td>1</td></tr></table>	CONTROL	CEA	1	1 x 0.75 mL	
CONTROL	CEA	1			
<table border="1"><tr><td>CONTROL</td><td>CEA</td><td>2</td></tr></table>	CONTROL	CEA	2	1 x 0.75 mL	
CONTROL	CEA	2			

Human CEA in a Tris-HCl buffered salt solution containing bovine serum albumin, and 0.01% methyl-isothiazolone (MIT) as preservative. Ready for use.

<b>BIOTIN</b>	<b>Anti-CEA</b>
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<b>Biotin Anti-CEA</b>	1 x 15 mL	2–8°C until expiry date stated on the vial
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Biotin Anti-CEA monoclonal antibody from mouse, approximately 3 µg/mL. Contains phosphate buffered saline (pH 7.2), bovine serum albumin, bovine immunoglobulin, blocking agents, Tween 20, an inert blue dye and 0.01% methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-CEA before use.

Component	Quantity	Storage and stability after first opening
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CONJ	Anti-CEA
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<b>Tracer, HRP Anti-CEA</b>	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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Stock solution of HRP Anti-CEA monoclonal antibody from mouse, approximately 60 µg/mL. Contains preservatives. To be mixed with Biotin Anti-CEA before use.

SUBS	TMB
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<b>TMB HRP-Substrate</b>	1 x 12 mL	2–8°C until expiry date stated on the vial
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Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.

STOP
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<b>STOP Solution</b>	1 x 15 mL	2–8°C until expiry date stated on the vial
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Contains 0.12 M hydrochloric acid. Ready for use.

WASHBUF	25X
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<b>Wash Concentrate</b>	1 x 50 mL	2–8°C until expiry date stated on the bottle
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A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative. To be diluted with water 25 times before use.

### Indications of instability

The TMB HRP-Substrate should be colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

## **WARNINGS AND PRECAUTIONS**

**For Research Use Only. Not for use in diagnostic procedures.**

- Please refer to the US Department of Health and Human Services (Bethesda, Md., US) publication No. (CDC) 88-8395 on laboratory safety or any other local or national regulation.
- Handle all serum specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

### **Caution**

Material used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV-1/2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

## **SPECIMEN COLLECTION AND HANDLING**

The CanAg CEA EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2–8° C for 2 days. For longer periods it is recommended to store the samples at –20°C or below. Avoid repeated freezing and thawing of the samples. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and then bring the samples to room temperature before analysis.

## **PROCEDURE**

### **Materials required but not supplied with the kit**

#### **1. Microplate shaker**

Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 700-900/min.

#### **2. Microplate wash device**

Automatic platewash capable of performing 1 and 6 washing cycles, or a semi manual microplate washing device connected to vacuum pump or water-jet vacuum and a liquid trap for retaining aspirated liquid.

The Nunc Immuno-8 manual strip washer is recommended if an automatic microplatewash is not used.

#### **3. Microplate spectrophotometer**

With a wavelength of 620 nm and/or 405 nm and an absorbance range of 0 to 3.0.

#### **4. Precision pipettes**

With disposable plastic tips to deliver microlitre and millilitre volumes. An 8-channel pipette or respenser pipette with disposable plastic tips for delivery of 100  $\mu$ L is useful but not essential.

#### **5. Distilled or deionized water**

For preparation of Wash Solution.

### **Procedural notes**

- 1.** A thorough understanding of this package insert is necessary to ensure proper use of the CanAg CEA EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
- 2.** Reagents should be allowed to reach room temperature (20–25°C) prior to use. The assay should only be performed at temperatures between 20–25°C to obtain accurate results. Frozen specimens should be brought to room temperature slowly and must be gently but thoroughly mixed after thawing.
- 3.** Before starting to pipette calibrators, controls and specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
- 4.** A careful washing procedure of the strips is essential. Ensure that each well is filled up completely to the top edge and that the aspiration of the wells between and after the washing cycles is complete and the wells are dry. If there is liquid left in the wells, invert the plate and tap it carefully against absorbing paper.

Automatic strip washer: Follow the manufacturer's instructions for maintenance and wash the required number of wash cycles prior to and after each incubation step.

The aspiration/wash device should not be left standing with the Wash Solution for long periods as the needles may get clogged, giving poor liquid delivery and suction.

- 5.** The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or respenser pipette tip).
- 6.** Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate Solution.

# Protocol Sheet

**CanAg CEA EIA** REF **401-85**

Mix the components directly before use. Use shaking conditions according to the Instructions.

Step	Bottle/Plate	Procedure			
1. Prepare Wash Solution	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.			
	CONJ Anti-CEA				
Prepare Antibody Solution	BIOTIN Anti-CEA	Mix 50 $\mu$ L of Tracer, HRP Anti-CEA, with 1 mL of Biotin Anti-CEA per strip:			
	<b>No. of Strips</b>	<b>Tracer, HRP Anti-CEA</b>	<b>Biotin Anti-CEA</b>	<b>(<math>\mu</math>L)</b>	<b>(mL)</b>
		1		50	1
		2		100	2
		3		150	3
		4		200	4
		5		250	5
		6		300	6
		7		350	7
		8		400	8
		9		450	9
		10		500	10



Preparation of reagents	Stability of prepared reagent
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**Wash Solution**

2 weeks at 2–25°C in a sealed container

Pour the 50 mL Wash Concentrate into a clean container and dilute 25- fold by adding 1200 mL of distilled or deionized water to give a buffered Wash Solution.

**Antibody Solution**

3 weeks at 2–8°C

Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-CEA with 1 mL of Biotin Anti-CEA per strip (see table below and the Protocol Sheet).

No. of Strips	Tracer, HRP Anti-CEA (µL)	Biotin Anti-CEA (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass bottle for preparation of the Antibody Solution.

**Alternative:** Pour the content of the Tracer, HRP Anti-CEA into the vial of Biotin Anti-CEA and mix gently. Make sure that all of the Tracer, HRP Anti-CEA is transferred to the vial of Biotin Anti-CEA.

**NOTE:** The Antibody Solution is stable for 3 weeks at 2–8°C. Do not prepare more Antibody Solution than will be used within this period and make sure that it is stored properly.

**Assay procedure**

Perform each determination in duplicate for calibrators, controls and unknown samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25°C) before use.

1. Start to prepare Wash Solution and Antibody Solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Pipette 25  $\mu\text{L}$  of the CEA Calibrators (CAL 0, 2, 5, 15, 50, 75), controls (c) and unknown samples (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal 0	Cal 50	Unk 1				
B	Cal 0	Cal 50	Unk 1				
C	Cal 2	Cal 75	Unk 2				
D	Cal 2	Cal 75	Unk 2				
E	Cal 5	C1	etc.				
F	Cal 5	C1					
G	Cal 15	C2					
H	Cal 15	C2					

4. Add 100  $\mu\text{L}$  of Antibody Solution to each well using a 100  $\mu\text{L}$  precision pipette (or an 8-channel 100  $\mu\text{L}$  precision pipette). Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid.
5. Incubate the frame containing the strips for 1 hour ( $\pm 5$  min) at room temperature (20–25°C) with constant shaking of the plate using a microplate shaker.
6. Wash each strip 6 times, using the wash procedure described in Procedural notes item 4.
7. Add 100  $\mu\text{L}$  of TMB HRP-Substrate to each well using the same pipetting procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between the addition to the first and last well should not exceed 5 min.

8. Incubate for 30 min ( $\pm$  5 min) at room temperature with constant shaking. Avoid direct sunlight.
9. Immediately read the absorbance at 620 nm in a microplate spectrophotometer.

### **Option**

If the laboratory does not have access to a microplate spectrophotometer capable of reading at 620 nm, the absorbance can be determined as follows:

- Alt. 9. Add 100  $\mu$ L of Stop Solution. Mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 minutes after addition of Stop Solution.

### **Measurement range**

The CanAg CEA EIA measures concentrations between 0.25 and 75  $\mu$ g/L. If CEA concentrations above the measuring range are to be expected, it is recommended to dilute samples with CEA Calibrator 0 prior to analysis.

### **Quality control**

CEA Control 1 and 2 may be used for validation of the assay series. Ranges of expected results are indicated on the vial labels. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated. It is recommended that each laboratory in addition prepare its own serum pools at different levels, which can be used as internal controls in order to assure the accuracy of the assay.

### **Reference material**

The 1<sup>st</sup> International Reference Preparation IRP 73/601 may be used as a reference standard. Values for CEA Calibrators and Controls were assigned against a set of in-house reference standards whose values are traceable to IRP 73/601 using the conversion factor 13.5, i.e. 1  $\mu$ g/L corresponds to 13.5 IU/L.

## CALCULATION OF RESULTS

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 CEA Calibrators.

For automatic calculation of CEA 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  $\mu\text{g/L}$ .
- 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  $\mu\text{g/L}$ .
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0  $\mu\text{g/L}$ .

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

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CEA calibrator against the corresponding CEA concentration (in  $\mu\text{g/L}$ ), see figure below. The unknown CEA concentrations can then be read from the calibration curve using the mean absorbance value of each specimen.

If samples in an initial analysis give CEA levels higher than 75  $\mu\text{g/L}$  the samples should be diluted 1/10 and 1/100 with CEA calibrator 0 and reanalyzed to obtain the accurate CEA concentration.

1 : 10 dilution = 50  $\mu\text{L}$  of specimen + 450  $\mu\text{L}$  of CEA 0  $\mu\text{g/L}$

1 : 100 dilution = 50  $\mu\text{L}$  of 1:10 dilution + 450  $\mu\text{L}$  of CEA 0  $\mu\text{g/L}$

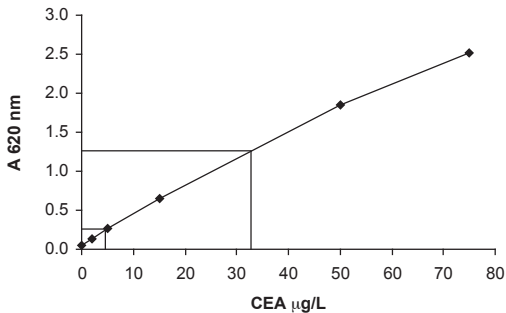
The CEA concentration of the undiluted sample is then obtained as follows:

Dilution 1/10: 10 x Measured value

Dilution 1/100: 100 x Measured value

## Example of results

Specimen			Calibrator values	Mean abs value (A)	CEA ( $\mu\text{g/L}$ )
CAL	CEA	0	0 $\mu\text{g/L}$	0.050	
CAL	CEA	2	2 $\mu\text{g/L}$	0.131	
CAL	CEA	5	5 $\mu\text{g/L}$	0.259	
CAL	CEA	15	15 $\mu\text{g/L}$	0.657	
CAL	CEA	50	50 $\mu\text{g/L}$	1.857	
CAL	CEA	75	75 $\mu\text{g/L}$	2.519	
Specimen A				0.220	4.1
Specimen B				1.290	32.3



Example (do not use this curve or table above to determine actual assay results).

## LIMITATIONS OF THE PROCEDURE

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer.

## WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by Fujirebio Diagnostics may affect the results, in which event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

## REFERENCES

1. Gold P. and Freedman S. O. (1965) Specific carcinoembryonic antigens of the human digestive system. *J. Exp Med* 122: 467–481.
2. Thompson J.A. and Zimmerman W. (1988) The carcinoembryonic antigen gene family: Structure, expression and evolution. *Tumor Biol*; 9: 63–83.
3. Börner, O.P. (1992) Thesis "Immunoassays for Carcinoembryonic antigen, Specificity and Interferences", ISBN 82-7633-014-2.
4. Hammarström S. et al. (1989) Antigenic sites in carcinoembryonic antigen. *Cancer Res* 49: 4852–4858.



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