

FOR INFORMATION ONLY.
WHEN PERFORMING
THE ASSAY ALWAYS REFER
TO PACKAGE INSERT
SUPPLIED
WITH THE KIT



CanAg CEA EIA

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

REF 401-85

Instructions for use. 2013-06



Distributed in the United States by:

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EN	EXPLANATION OF SYMBOLS
BG	ОБЯСНЕНИЕ НА СИМВОЛИТЕ
CS	VÝZNAM SYMBOLŮ
DA	SYMBOLFORKLARING
DE	ERKLÄRUNG DER SYMBOLE
EL	ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
ES	SIGNIFICADO DE LOS SÍMBOLOS
ET	SÜMBOLITE SELGITUS
FR	EXPLICATION DES SYMBOLES
HR	OBJAŠNJENJE SIMBOLA
HU	JELMAGYARÁZAT
IT	SPIEGAZIONE DEI SIMBOLI
LT	SIMBOLIŲ PAAIŠKINIMAI
LV	SIMBOLU SKAIDROJUMS
NL	VERKLARING DER SYMBOLEN
NO	SYMBOLFORKLARING
PL	OBJAŚNIENIE SYMBOLI
PT	EXPLICAÇÃO DOS SÍMBOLOS
RO	SEMNIȚAȚIA SIMBOLURILOR
RU	ОБОЗНАЧЕНИЯ
SV	SYMBOLFÖRKLARING
SK	VÝZNAM SYMBOLOV
SL	RAZLAGA SIMBOLOV
SR	OBJAŠNJENJE SIMBOLA
TR	SEMBOLLERİN AÇIKLAMALARI



Use Ву/Годно до/Použitelné do/
Holdbar til/Verwendbar bis/
Ημερομηνία λήξης/Fecha
de caducidad/Kölblük kuni/
Utiliser jusque/Rok valjanosti/
Felhasznáható/Utilizzare entro/
Sunaudoti iki/Izlietot līdz/Houdbaar
tot/Brukes innen/Uzyc przed/
Prazo de validade/Expirã la/
Использовать до/Använd före/
Použite né do/ Uporabno do/
Upotrebljivo do/Son Kullanna Tarihi

LOT

Batch code/Номер на партида/
Číslo šarže/Lotnummer/
Chargenbezeichnung/Αριθμός
Παρτίδας/Código de lote/Partii
kood/Code du lot/Kod serije/
Sarzsám/Codice del lotto/
Partijos kodus/Partijas kods/Lot
nummer/Partikode/Kod partii/
Código do lote/Număr de lot/
Номер лота/Lotnummer/Číslo
šarže/Številka serije/Kod partije/
Parti Kodu



Date of manufacture/Dاتا на производство/Datum výroby/
Produktionsdato/Herstellungsdatum/
Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/
Date of manufacture/Datum proizvodnje/
Gyártási idő/Data di produzione/
Pagaminimo data/Ražošanas datums/
Productiedatum/Fremstillingsdato/
Data produkcyj/Data de fabrico/Data fabricației/Data производства/
Tillverkningsdatum/Datum výroby/Datum izdelave/Datum proizvodnje/Ûretim tarihi



Temperature limitation/
Температури граници/
Teplotní omezení/
Temperaturbegrensning/
Temperaturbegrenzung/
Περιορισμοί θερμοκρασίας/
Limites de temperatura/
Limites de temperatura/
Limite de température/
Temperaturograničenje/
Hőmérsékletre vonatkozó korlátozások/
Limiti di temperatura/
Temperatūriņa ierobežojumi/
Temperatūras ierobežojums/
Temperaturbepörking/
Temperaturbegrensninger/
Temperaturgraniczne/
Limite de temperatură/
Limite de temperaturá/
Температурный режим/
Temperaturbegrensning/
Teplotné obmedzenie
Omejitve temperature/
Temperaturograničenje/
Sıcaklık sınırlaması

IVD

In Vitro Diagnostic Medical Device/
Медицински уред за диагностика
ин vitro/Diagnostický zdravotnícký
prostředek in vitro/Medicinsk udstyr til
in vitro-diagnostik/In-vitro-Diagnostikum/
Ιατροτεχνολογικό προϊόν για διάγνωση
In Vitro/Dispositivo médico para
diagnóstico in vitro/In vitro diagnostiline
meditsiiniseade/Dispositif médical de
diagnostic in vitro/Diagnostički medicinski
uređaj In Vitro/In vitro orvodnosniotkai
eszköz/Dispositivo medico per test
diagnostici in vitro/In Vitro Diagnostinė
Medicinos Priemonė/Medicinska ierīce
in vitro diagnostiskai/In vitro-diagnostisch
medisch instrument/In vitro diagnostisk
medisinsk udstyr/Wyrób medyczny do
diagnostyki in vitro/Dispositivo Médico
de Diagnóstico In Vitro/Dispozitiv medical
pentru diagnostic in vitro/Только для
диагностики In Vitro/Endast för in
vitro-diagnostik/ Zdravotnicka pomôcka na
diagnostiku in vitro/In vitro diagnostični
pripomoček/Diagnostički medicinski
uređaj In Vitro/<96> testleri için yeterlilik
içerir



Contains sufficient for <96> tests/Съдържа
достатъчно количество за тестове
<96>/Lež použít pro <96> testů/Ineholder
tilstrækkeligt/Inhalt ausreichend für <96>
Prüfungen/Περιεχόμενο επαρκές για
<96> εξετάσεις/Contenido suficiente para
<96> ensayos/Koguseisut sisab <96> testi
läbimiseks/Contenu suffisant pour <96>
testuri/Sadržaj dovoljno za <96> testova/A
doboz tartalma <96> vizsgálat elvégzéséhez
elegendő/Contenuto sufficiente per <96>
saggi/Turiny skirtas atliki <96> tyrimus/
Saturis pietiekams <96> testiem/Inhoud
voldoende voor <96> testen/til <96> test/
Tilstrækkelig innhold for <96> prøver/
Wystarczy na wykonanie <96> testów/
Conteúdo suficiente para <96> ensaios/
Conținut suficient pentru 96 de teste/
Содержит достаточное количество для
<96> определений/Innehåller tillräckligt
till <96> antal tester/Obsah postařuje na
tento počet testov. <96>/Vsebina zadostuje
za <96> testov/Sadržina dovoljna za <96>
testova/<96> testleri için yeterlilik içerir

REF

Catalogue number/Κατάλογος номер/
Katalogové číslo/Katalognummer/
Bestellnummer/Αριθμός καταλόγου/
Número de catálogo/Kataloogi number/
Número de catalogue/Kataloški broj/
Katalógusszám/Numero di catalogo/
Katalogo numeris/Numurs katalogā/
Catalogusnummer/Katalognummer/
Numer katalogowy/Numero do catálogo/
Număr de catalog/Номер по каталогу/
Produktnummer/Katalógové číslo/
Kataloška številka/Kataloški broj/
Katalog numarası



Consult Instructions for Use/
Прочетете инструкцията за
употреба/Konzultujte s návodom
k použití/Se brugsanvisning/Siehe
Gebrauchsanweisung/Συμβουλευτείτε
τις Οδηγίες σχετική με τη χρήση/
Consulte las instrucciones de uso/
Vi kasutusjuhendit/Consulter le mode
d'emploi/Pročitajte upute za uporabu/
Olvasa el a használati utasítást/
Consultare le istruzioni per l'uso/Dél
naudojimo židėkite instrukcijas/Izlasiet
lietošanas instrukciju/Raadpleeg de
instructions voor gebruik/Les instruksene
fer bruk/Sprawdź w instrukcji użycia/
Consulte as Instruções de Utilização/
Consultati instrucțiunile de utilizare/
Обратитесь к инструкции по
применению/Se bruksanvisning/
Prečítajte si návod na používanie/
Pročitajte uputstvo za upotrebu/
Kullanım Talimatlarını Bakınız

CONT

Contents of kit/Съдържание на набора/
Obsah soupravy/Kittets indhold/Inhalt
des Kits/Περιεχόμενα του κιτ/Contenido
del kit/Komplekt sisältab/Contenu du
kit/Sadržaj opreme/A készlet tartalma/
Contenuto del kit/Rinkinio turinys/
Komplekta saturis/Inhoud van de set/
Settets innhold/Zawartość zestawu/
Conteúdo do kit/Conținutul setului/
Компоненти набора/Kit innehåll/
Obsah súpravy/Vsebina kompleta/Sadržaj
opreme/Kitin iņėdikler



Biological risks/Биологическа
опасност/Biológická rizika/Biologisk
fare/Biologische Gefahren/Biológyci
kivndvonal/Riesgos biológicos/
Biologilised ohud/Risques biologiques/
Biološki rizici/Biológiai kockázatok/Rischi
biologici/Biológinis pavojus/Biológiskais
risks/Biologische risico's/Biologiske
risikoer/Zagrożenie biologiczne/Riscos
biológicos / Biologisk risk/Pericole
biologice/Биологическая опасность/
Biologicky rizikové/Biológické riziká/
Biološki rizici/Bjyolójik risler

ORIG HUM

Human/C човешки производ/Lidské/
Human/Human/δείγματα αναφοράς/
Humano/Inimāritulo/Humaine/Ljudskog
porijekla/Human/Humán/Origine Umana/
Žmogaus kilmės/Cilvēku izcelsmes/
Human/Menneske/Ludzka/Humano/
Origine umană/Человеческого
происхождения/Human/Lidské/
Humaneга izvora/Ljudskog porijekla/İnsan

ORIG MOU

From mouse/C миши производ/Mušji/
Fra mus/Maus/отто повитки/de ratón/
Hiirtelt/De souris/Mišijeg porjekla/
Egérből/Muniro/Peles kilmės/No peles/
Van muizen/Fra mus/Mysia/Do rato/De
la șoareci/Мышиного происхождения/
Från mus/Mýšje/Mišjega izvora/Mišijeg
porjekla/Fareden

ORIG BOV

Bovine/C говежди производ/
Hovězí/Bovin/Rind/отб βοοειδή/
Bovino/Veistelt/Bovine/Rogate stoke/
Szarvasmarha/Bovino/Jaučilo/No
liellopa/Bovien/Bovin/Wolowy/Bovino/
Origine bovină/крупного рогатого
скота/Från ko/Hovdzíe/Govejega
izvora/Rogate krupne stoke/Bovin



Reconstituted with/Пастваряние c/
Rožfede pomocil/Rekonstitues med/
Rekonstituieren mit/Ανασύσταση με/
Reconstituir con/Lahjendamine/
Reconstituer avec/Rekonstituirtaje s/
Feloldashoz/Reconstituire con/Atkurti,
ištirpdano su/Ricșkaidit ar/Reconstitutie
met/Rekonstituieres med/Otdworzyć
za pomocą/Reconstituir com/A
se reconstitui cu/Пастворить в/
Rekonstituira med/Rozfiedite pomocou/
Rekonstituirtaje z/s/Ponovno formiranje
sa/Yeniden oluşturulur



Manufacturer/Производитель/Výrobce/
Producent/Hersteller/Κατασκευστής/
Fabricante/Tootja/Fabricant/Proizvođač/
Gyártó/Fabbricante/Gamintojas/
Roztočís/Fabrikant/Produsent/
Producent/Fabricante/Producător/
Производитель/Tillverkare/ Výrobca/
Izdevalavec/Proizvođač/Ûretici

CanAg CEA EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

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
-----------	----------	---

CEA Calibrators	6 vials	2–8° C until expiry date stated on the vials
CAL CEA 0	0 µg/L	1 x 8 mL
CAL CEA 2	2 µg/L	1 x 0.75 mL
CAL CEA 5	5 µg/L	1 x 0.75 mL
CAL CEA 15	15 µg/L	1 x 0.75 mL
CAL CEA 50	50 µg/L	1 x 0.75 mL
CAL CEA 75	75 µg/L	1 x 0.75 mL

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.

Component	Quantity	Storage and stability after first opening
CEA Controls	2 vials	2–8° C until expiry date stated on the vials
CONTROL CEA 1	1 x 0.75 mL	
CONTROL CEA 2	1 x 0.75 mL	

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

BIOTIN | Anti-CEA

Biotin Anti-CEA 1 x 15 mL 2–8° C until expiry date stated on the vial

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.

CONJ | Anti-CEA

Tracer, HRP Anti-CEA 1 x 0.75 mL 2–8° C until expiry date stated on the vial

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

TMB HRP-Substrate 1 x 12 mL 2–8° C until expiry date stated on the vial

Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.

Component	Quantity	Storage and stability after first opening
STOP		
STOP Solution	1 x 15 mL	2–8° C until expiry date stated on the vial

Contains 0.12 M hydrochloric acid. Ready for use.

WASHBUF | 25X

Wash Concentrate 1 x 50 mL 2–8° C until expiry date stated on the bottle

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, approximately 700-1100 oscillations/min.

2. **Microplate wash device**

Automatic plate washer capable of performing 1 and 6 washing cycles with a minimal fill volume of 350 µL/well/washcycle.

An 8-channel pipette with disposable plastic tips for delivery of 350 µL is recommended if an automatic microplate washer 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 µL 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. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. In order to ensure efficient washing make sure that all wells are completely filled to the top edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.
 - Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. It's highly recommended to use *strip* process mode and *overflow* wash mode with a dispensing volume of 800 µL. The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.
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	Vial/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 µL of Tracer, HRP Anti-CEA, with 1 mL of Biotin Anti-CEA per strip:																																							
	Anti-CEA																																								
		<table border="1"> <thead> <tr> <th>No. of Strips</th> <th>Tracer, HRP Anti-CEA (µL)</th> <th>Biotin Anti-CEA (mL)</th> </tr> </thead> <tbody> <tr><td>1</td><td>50</td><td>1</td></tr> <tr><td>2</td><td>100</td><td>2</td></tr> <tr><td>3</td><td>150</td><td>3</td></tr> <tr><td>4</td><td>200</td><td>4</td></tr> <tr><td>5</td><td>250</td><td>5</td></tr> <tr><td>6</td><td>300</td><td>6</td></tr> <tr><td>7</td><td>350</td><td>7</td></tr> <tr><td>8</td><td>400</td><td>8</td></tr> <tr><td>9</td><td>450</td><td>9</td></tr> <tr><td>10</td><td>500</td><td>10</td></tr> <tr><td>11</td><td>550</td><td>11</td></tr> <tr><td>12</td><td>600</td><td>12</td></tr> </tbody> </table>	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
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																																							
2. Wash	MICROPLA	Wash each well once with wash solution																																							
3. Add calibrators, controls and samples	CAL CEA	25 µL in each well																																							
	0, 2, 5, 15, 50, 75																																								
	CONTROL CEA																																								
	1, 2																																								
4. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well																																							
5. Incubate	MICROPLA	1 hour shaking at room temperature																																							
6. Wash	MICROPLA	Wash each well six times with wash solution																																							
7. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well																																							
8. Incubate	MICROPLA	30 min shaking at room temperature																																							
9. Read absorbance	MICROPLA	620 nm																																							
Alt.9 Add Stop Solution	STOP	100 µL in each well																																							
Alt.10 Incubate	MICROPLA	1 min shaking at room temperature																																							
Alt.11 Read absorbance	MICROPLA	Read at 405 nm within 15 min																																							

Preparation of reagents	Stability of prepared reagent
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
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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 µL 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 µL of Antibody Solution to each well using a 100 µL precision pipette (or an 8-channel 100 µL 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 (± 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 µL 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 μ 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 μ 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 1st 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 μ 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 μ 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 μ g/L.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 μ 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 μ 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 μ 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 μ L of specimen + 450 μ L of CEA 0 μ g/L

1 : 100 dilution = 50 μ L of 1:10 dilution + 450 μ L of CEA 0 μ g/L

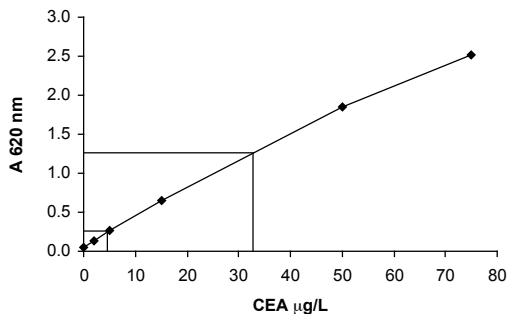
The CEA concentration of the undiluted sample is then obtained as follows:

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.

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