CanAg CA242 EIA

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

REF 101-85

Instructions for use. 2022-06

Read highlighted changes

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	SEMNIFICAŢIA SIMBOLURILOR
RU	ОБОНАЧЕНИЯ
SV	SYMBOLFÖRKLARING
SK	VÝZNAM SYMBOLOV
SL	RAZLAGA SIMBOLOV
SR	OBJAŠNJENJE SIMBOLA
TR	SEMBOLLERİN AÇIKLAMALARI

Use By/Годно до/Použitelné do/Holdbar til/Verwendbar bis/Ημερομηνία λήξης/Fecha de caducidad/Kõlblik kuni/Utiliser jusque/Rok valjanosti/Felhasználható/Utilizzare entro/Sunaudoti iki/Izlietot līdz/Houdbaar tot/Brukes innen/Użyć przed/Prazo de validade/Expiră la/Использовать до/Använd före/Použite´né do/ Uporabno do/Upotrebljivo do/Son Kullanma Tarihi

LOT

Batch code/Номер на партида/Číslo šarže/Lotnummer/Chargenbezeichnung/Αριθμός Παρτίδας/Código de lote/Partii kood/Code du lot/Kod serije/Sarzsszám/Codice del lotto/Partijos kodas/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/Дата на производство/Datum výroby/Produktionsdato/Herstellungsdatum/Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/Date de fabrication/Datum proizvodnje/Gyártási idő/Data di produzione/Pagaminimo data/Ražošanas datums/Productiedatum/Fremstillingsdato/Data produkcji/Data de fabrico/Data fabricaţiei/Дата производства/Tillverkningsdatum/Dátum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi

IVD

In Vitro Diagnostic Medical Device/Медицински уред за диагностика ин витро/Diagnostický zdravotnický 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 orvosdiagnosztikai eszköz/Dispositivo medico per test diagnostici in vitro/In Vitro Diagnostinė Medicinos Priemonė/Medicīniska ierīce in vitro diagnostikai/In vitro-diagnostisch medisch instrument/In vitro diagnostisk medisinsk utstyr/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/ Zdravotnícka pomôcka na diagnostiku in vitro/In vitro diagnostični pripomoček/Diagnostički medicinski uređaj In Vitro/<96> testleri için yeterlilik içerir

REF

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

Temperature limitation/Температурни граници/Teplotní omezení/Temperaturbegrænsning/Temperaturbegrenzung/Περιορισμοί θερμοκρασίας/Límites de temperatura/Temperatuuri piirang/Limite de température/Temperaturno ograničenje/Hőmérsékletre vonatkozó korlátozás/Limiti di temperatura/Temperatūriniai apribojimai/Temperatūras ierobežojums/Temperatuurbeperking/Temperaturbegrensninger/Temperatury graniczne/Limite de temperatura/Limite de temperatură/Температурный режим/Temperaturbegränsning/Teplotné obmedzenie Omejitev temperature/Temperaturno ograničenje/Sıcaklık sınırlaması/

Σ

Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Indeholder tilsttrækkeligt/Inhalt ausreichend für <96> Prüfungen/Περιεχόμενο επαρκές για «96» εξετάσεις/Contenido suficiente para <96> ensayos/Kogusest piisab <96> testi läbiviimiseks/Contenu suffisant pour “96” tests/Sadrži dovoljno za <96> testova/A doboz tartalma <96> vizsgálat elvégzéséhez elegendő/Contenuto sufficiente per “96” saggi/Turinys skirtas atlikti <96> tyrimus/Saturs pietiekams <96> testiem/Inhoud voldoende voor “96” testen/til “96” test/Tilstrekkelig innhold for <96> prøver/Wystarczy na wykonanie <96> testów/Conteúdo suficiente para “96” ensaios/Conţinut suficient pentru 96 de teste/Содержит достаточные количества для «96» определений/Innehåller tillräckligt till “96” antal tester/Obsah postačuje na tento počet testov: <96>/Vsebina zadostuje za <96> testov/Sadržina dovoljna za <96> testova/<96> testleri için yeterlilik içerir

Consult Instructions for Use/ Прочетете инструкцията за употреба/Konzultujte s návodem k použití/Se brugsanvisning/Siehe Gebrauchsanweisung/Συμβουλευτείτε τις Οδηγίες σχετικά με τη χρήση/ Consulte las instrucciones de uso/ Vt kasutusjuhendit/Consulter le mode d'emploi/Pročitajte upute za uporabu/ Olvassa el a használati utasítást/ Consultare le istruzioni per l'uso/Dėl naudojimo žiūrėkite instrukcijas/Izlasiet lietošanas instrukciju/Raadpleeg de instructies voor gebruik/Les instruksene før bruk/Sprawdzić w instrukcji użycia/ Consulte as Instruções de Utilização/ Consultaţi instrucţiunile de utilizare/ Обратитесь к инструкции по применению/Se bruksanvisning/ Prečítajte si návod na používanie/ Pročitajte uputstvo za upotrebu/ Kullanım Talimatlarına Bakınız

CONT

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

Biological risks/Биологическа опасност/Biologická rizika/Biologisk fare/Biologische Gefahren/Βιολογικοί κίνδυνοι/Riesgos biológicos/ Bioloogilised ohud/Risques biologiques/ Biološkli rizici/Biológiai kockázatok/Rischi biologici/Biologinis pavojus/Bioloģiskais risks/Biologische risico's/Biologiske risikoer/Zagrożenie biologiczne/Riscos biológicos/ Biologisk risk/Pericole biologice/Биологическая опасность/ Biologicky rizikové/Biologické riziká/ Biološkli rizici/Biyolojik riskler

ORIG **HUM**

Human/C човешки произход/Lidské/ Humant/Human/δείγματα αναφοράς/ Humano/Inimpäritolu/Humaine/Ljudskog porjekla/Humán/Origine Umana/ Žmogaus kilmės/Cilvēku izcelsmes/ Human/Menneske/Ludzka/Humano/ Origine umană/Человеческого происхождения/Human/Ľudské/ Humanega izvora/Ljudskog porekla/İnsan

ORIG **MOU**

From mouse/C миши произход/Myší/ Fra mus/Maus/από ποντίκι/de ratón/ Hiirtelt/De souris/Mišijeg porjekla/ Egérből/Murino/Pelės kilmės/No peles/ Van muizen/Fra mus/Mysia/Do rato/De la şoareci/Мышиного происхождения/ Från mus/Myšie/Mišjega izvora/Mišijeg porekla/Fareden

ORIG **BOV**

Bovine/C говежди произход/ Hovězí/Bovin/Rind/από βοοειδή/ Bovino/Veistelt/Bovine/Rogate stoke/ Szarvasmarha/Bovino/Jaučio/No liellopa/Bovien/Bovin/Wołowy/Bovino/ Origine bovină/крупного рогатого скота/Från ko/Hovädize/Govejega izvora/Rogate krupne stoke/Bovin

Reconstitute with/Разтваряне с/ Rozřeďte pomocí/Rekonstitueres med/ Rekonstituieren mit/Ανασύσταση με/ Reconstituir con/Lahjendamine/ Reconstituer avec/Rekonstituirajte s/ Feloldáshoz/Ricostituire con/Atkurti, ištirpdant su/Atšķaidīt ar/Reconstitutie met/Rekonstitueres med/Odtworzyć za pomocą/Reconstituir com/A se reconstitui cu/Растворить в/ Rekonstituera med/Rozrieďte pomocou/ Rekonstituirajte z/s/Ponovno formiranje sa/Yeniden oluşturulur

Manufacturer/Производител/Výrobce/ Producent/Hersteller/Κατασκευαστής/ Fabricante/Tootja/Fabricant/Proizvođač/ Gyártó/Fabbricante/Gamintojas/ Ražotājs/Fabrikant/Produsent/ Producent/Fabricante/Producător/ Производитель/Tillverkare/ Výrobca/ Izdelovalec/Proizvođač/Üretici

EN

CanAg CA242 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg CA242 EIA kit is intended for the quantitative determination of CA242 cancer antigen in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The tumor marker CA242 is defined by the monoclonal antibody C242. The chemical structure of the antigenic determinant is not exactly known, but the determinant have been shown to be a sialylated carbohydrate structure. In serum, CA242 is found on the same mucin-complex as CA50 and sialylated Lewis^a (CA19-9). Thus, CA242 is related, but not identical to the epitope of CA19-9 (1, 2).

PRINCIPLE OF THE TEST

The CanAg CA242 EIA is a solid-phase, non-competitive immunoassay based on the direct sandwich technique. Calibrators, controls and samples are incubated together with biotinylated anti-CA242 monoclonal antibody (MAb) C241 in Streptavidin coated microstrips. CA242 present in calibrators, controls or samples is adsorbed to the Streptavidin coated microstrips by the biotinylated anti-CA242 MAb during the incubation (3). The strips are then washed and incubated with horseradish peroxidase (HRP) labelled Anti-CA242 MAb C242. 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 CA242 antigen 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 CA242 concentrations of samples are then read from the calibration curve.

REAGENTS

- Each CanAg CA242 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
MICROPLA		
Microplate	1 Plate	2–8° C until expiry date stated on the plate

12 x 8 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

CA242 Calibrators			5 vials	2–8° C until expiry date stated on the vials
CAL	CA242	0	0 U/mL 1 x 0.75 mL	
CAL	CA242	15	15 U/mL 1 x 0.75 mL	
CAL	CA242	50	50 U/mL 1 x 0.75 mL	
CAL	CA242	100	100 U/mL 1 x 0.75 mL	
CAL	CA242	150	150 U/mL 1 x 0.75 mL	

Human CA242 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye, and 0.05 % NaN_3 as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
CA242 Controls	2 vials	2–8° C until expiry date stated on the vials
CONTROL | CA242 | 1	1 x 0.75 mL	
CONTROL | CA242 | 2	1 x 0.75 mL	

Human CA242 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, and 0.05 % NaN_3 as preservative. Ready for use.

BIOTIN	Anti-CA242

Biotin Anti-CA242	1 x 15 mL	2–8° C until expiry date stated on the vial

Biotin Anti-CA242 monoclonal antibody from mouse, approximately 1.5 µg/mL. Contains Tris-HCl buffered saline (pH 7.75) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert red dye and 0.05 % NaN_3 as preservative. Ready for use.

CONJ	Anti-CA242

Tracer, HRP Anti-CA242	1 x 0.75 mL	2–8° C until expiry date stated on the vial

Stock solution of HRP Anti-CA242 monoclonal antibody from mouse, approximately 40 µg/mL. Contains preservatives. To be diluted with Tracer Diluent prior to use.

DIL	CONJ

Tracer Diluent	1 X 15 mL	2–8° C until expiry date stated on the vial

Phosphate buffered saline (pH 7.2) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert blue dye, and 0.01 % methylisothiazolone (MIT) as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
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.		
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 specimens as potentially infectious.
- Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
- 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.

CLP (1272/2008) HAZARD CLASSIFICATION

Information about CLP (1272/2008) HAZARD CLASSIFICATION can be found at the end of this document.

SPECIMEN COLLECTION AND HANDLING

The CanAg CA242 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 24 hours. 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, 3 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 CA242 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 (20–28° C) prior to use. The assay should only be performed at temperatures between 20–28° 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.

Protocol Sheet

CanAg CA242 EIA REF 101-85

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 or deionized water.
	Prepare Tracer working solution	CONJ Anti-CA242 DIL CONJ	Mix 50 µL of Tracer, HRP Anti-CA242 with 1mL of Tracer Diluent per strip:
2.	Wash	MICROPLA	Wash each well once with Wash Solution. Use manual or automatic washer.

No. of Strips	Tracer, HRP Anti-CA242 (µL)	Tracer Diluent (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

3.	Add calibrators, controls and samples	CAL CA242 0, 15, 50, 100, 150 CONTROL CA242 1, 2	25 µL in each well
4.	Add Biotin Anti-CA242	BIOTIN Anti-CA242	100 µL in each well
5.	Incubate	MICROPLA	2 hours shaking at room temperature
6.	Wash	MICROPLA	Wash each well three times with Wash Solution. Use manual or automatic washer.
7.	Add Tracer working solution	TRACER WORKING SOLUTION	100 µL in each well
8.	Incubate	MICROPLA	1 hour shaking at room temperature
9.	Wash	MICROPLA	Wash each well six times with Wash Solution. Use manual or automatic washer.
10.	Add TMB HRP-Substrate	SUBS TMB	100 µL in each well
11.	Incubate	MICROPLA	30 min shaking at room temperature
12.	Read absorbance	MICROPLA	620 nm
Alt.12	Add Stop Solution	STOP	100 µL in each well
Alt.13	Incubate	MICROPLA	1 min shaking at room temperature
Alt.14	Read absorbance	MICROPLA	Read at 405 nm within 5 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.

Tracer Working Solution	3 weeks at 2–8° C in a sealed container

Prepare the required quantity of Tracer working solution by mixing 50 µL of Tracer, HRP Anti-CA242 with 1 mL of Tracer Diluent per strip (see table below):

No. of Strips	Tracer, HRP Anti-CA242 (µL)	Tracer Diluent (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 Tracer Working Solution.
Alternative: Pour the content of the Tracer, HRP Anti-CA242 into the vial of Tracer Diluent and mix gently. Make sure that all of the Tracer, HRP Anti-CA242 is transferred to the vial of Tracer Diluent.
NOTE: The Tracer Working Solution is stable for 3 weeks at 2–8° C. Do not prepare more Tracer Working 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 samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–28° C) before use.

1. Start to prepare Wash Solution and Tracer Working 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 CA242 Calibrators (CAL 0, 15, 50, 100, 150), Controls (C1, C2) and samples (unknowns Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal 0	Cal 150	Unk 2				
B	Cal 0	Cal 150	Unk 2				
C	Cal 15	C1	etc.				
D	Cal 15	C1					
E	Cal 50	C2					
F	Cal 50	C2					
G	Cal 100	Unk 1					
H	Cal 100	Unk 1					

4. Add 100 µL of Biotin Anti-CA242 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 2 hours (± 10 min) at room temperature (20–28° C) with constant shaking of the plate using a microplate shaker.
6. After the first incubation aspirate and wash each strip 3 times using the wash procedure described in Procedural notes, item 4.
7. Add 100 µL of Tracer working solution to each well. Use the same pipetting procedure as in item 4 above.
8. Incubate the frame for 1 hour (± 5 min) at room temperature (20–28° C) with constant shaking.

9. After the second incubation aspirate and wash each strip 6 times, using the wash procedure described in Procedural notes, item 4.
10. 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.
11. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.
12. 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. 12. Add 100 µL of Stop Solution. Mix and read the absorbance at 405 nm in a microplate spectrophotometer within 5 minutes after addition of Stop Solution.

Measurement range

The CanAg CA242 EIA measures concentrations between 1 and 150 U/mL. If CA242 concentrations above the measuring range are to be expected, it is recommended to dilute samples with normal human serum prior to analysis. **NOTE:** The serum used for dilution must also be measured in order to determine the endogenous CA242 concentration (see “Calculation of results”).

Quality control

CA242 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. Each laboratory may in addition prepare its own serum pools at different levels, which can be used as internal controls in order to assure the precision of the assay.

Reference material

Since no common reference material is available for CA242 antigen, CanAg CA242 EIA Calibrator values are assigned against a set of in-house reference standards.

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 CA242 Calibrators.
For automatic calculation of CA242 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 U/mL.
- 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 U/mL.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 U/mL.

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 CA242 Calibrator against the corresponding CA242 concentration (in U/mL), see figure below. The unknown CA242 concentrations can then be read from the calibration curve using the mean absorbance value of each specimen.

If samples in an initial analysis give CA242 levels higher than 150 U/mL, the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate CA242 concentration. **NOTE**: The sample used for dilution must also be measured in order to determine the endogenous CA242 concentration.
The CA242 concentration of the undiluted sample is calculated as:

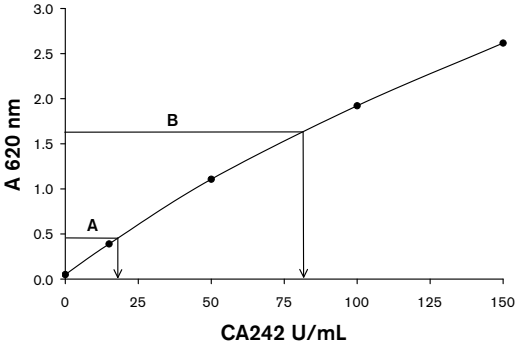
Dilution 1/10: $10 \times ([CA242]_{\text{Diluted sample}} - (0.9 \times [CA242]_{\text{Normal serum}}))$

Example of results

Specimen	Calibrator values	Mean abs value (A)	CA242 (U/mL)
CAL CA242 0	0 U/mL	0.050	
CAL CA242 15	15 U/mL	0.390	
CAL CA242 50	50 U/mL	1.107	
CAL CA242 100	100 U/mL	1.922	
CAL CA242 150	150 U/mL	2.617	
Specimen A		0.410	16.1
Specimen B		1.636	80.9

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.



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

CLP (1272/2008) HAZARD CLASSIFICATION

The following warnings and precautions apply to SUBS TMB

Hazard pictograms:

Signal word:	Danger
Hazard Statement:	Repr. 1B: H360D May damage the unborn child.
Prevention statement:	P202 Do not handle until all safety precautions have been read and understood.
Prevention:	P280 Wear protective gloves / protective clothing / eye protection / face protection.
Precautionary statement response:	P308+P313 IF exposed or concerned get medical advice/attention.
Precautionary statement distposal:	P501 Dispose of contents / container to an approved hazardous / special waste disposal facility in accordance with local and national regulations.

Restricted to professional users.

Hazardous substances: 2- Pyrrolidone

Other hazards

None of the mixtures in the kit contains any substances considered to meet the criteria classifying them as PBT and/or vPvB.

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

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2. Johansson, C., Nilsson, O. and Lindholm, L., (1991) Comparison of Serological Expression of Different Epitopes on the CA50-Carrying Antigen CanAg, *Int. J. Cancer,* 48, 757-763.
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