

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



CanAg NSE EIA

REF

420-10

IVD



Instructions for use. 2009-11

DE Wenden Sie sich bitten an die deutsche Niederlassung um die geltende Gebrauchsanweisung zu erhalten.

ES Por favor contacte con su distribuidor para una versión válida de "Instrucciones de uso" en español

IT Contattare il proprio Distributore per ottenere la versione ufficiale della traduzione in lingua Italiana delle Istruzioni per l'Uso

FR Pour une version certifiée de la Notice en Français, veuillez contacter votre Distributeur.

DK Kontakt venligst den danske distributør for gældende version af dansk brugsanvisning.

GR Παρακαλούμε όπως επικοινωνήσετε με τον προμηθευτή σας για την έγκυρη απόδοση στα Ελληνικά των οδηγιών χρήσης

SE Vänligen kontakta Er distributör för gällande version av bruksanvisning på svenska.

GB EXPLANATION OF SYMBOLS
DE BEDEUTUNG DER SYMBOLE
ES EXPLICACIÓN DE SÍMBOLOS
IT SIGNIFICATO DEI SIMBOLI
FR EXPLICATION DES SYMBOLES
NL PICTOGRAMMEN
DK SYMBOLFORKLARING
CS VYSVĚTLENÍ SYMBOLŮ
GR ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
PT INTERPRETAÇÃO DE SÍMBOLOS
HU JELMAGYARÁZAT
SE SYMBOLFÖRKLARING
PL INTERPRETACJA SYMBOLI
LT SIMBOLIŲ PAAIŠKINIMAI
RU ОБОЗНАЧЕНИЯ



Use By/Verwendbar bis/
Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Houdbaar tot/Holdbar til/
Ρουζιτελνή до/Ημερομηνία λήξης/
Prazo de validade/Felhasználható
Bäst före datum/Узгыч przed/
Sunaudoti iki/Использовать до

LOT

Batch code/
Chargenbezeichnung/
Codigo de lote/
Codice del lotto/Code du lot/
Lot number/Lotnummer/
Číslo šarže/Αριθμός Παρτίδας/
Código do lote/Sarzszzám
Lotnummer/Kod partii/Partijos
koda/Номер лота



Date of manufacture/
Herstellungsdatum/
Fecha de fabricación/
Data di fabbricazione/
Date of fabrication/
Produktie datum/Produktionsdato/
Datum výroby/Ημερομηνία
Παράγωγής/Data de fabrico/
Gyártás időpontja/Tillverkningsdatum/
Data produkcji/Pagaminimo data/
Дата производства

REF

Catalogue number/Bestellnummer/
Número de catálogo/
Numero di catalogo/Référence du
catalogue/Catalogus nummer/Katalog-
nummer/Katalogové číslo/
Αριθμός καταλόγου/
Referència de catálogo/
Katalógusszám/Produktnummer/
Numer katalogowy/Katalogo numeris/
Номер по каталогу



Manufacturer/Hersteller/Fabricante/
Fabbicante/Fabrant/Fabrikant/
Producent/Výrobce/Κατασκευαστής/
Fabricante/Gyártó/Tillverkare/
Producent/Gamintojas/
Производитель



Contains sufficient for <96> tests/
Inhalt ausreichend für <96> Prüfungen/
Contenido suficiente para <96>
ensayos/Contenuto sufficiente per
"96" saggi/Contenu suffisant pour
"96" tests/Inhoud voldoende voor "96"
testen/Indeholder tilstrækkeligt
til "96" test/Lze použit pro <96> testů/
Περιεχόμενο επαρκές για «96»
εξετάσεις/Conteúdo suficiente para
"96" ensaios/A doboz tartalma <96>
vizsgálat elvégzéséhez elegendő/
Innehåller tillräckligt till "96" antal tester/
Wystarczy na wykonanie <96> testów/
Turinys skirtas atlikti <96> tyrimus
/Содержит достаточные количества
для «96» определений



In Vitro Diagnostic Medical Device/
In Vitro Diagnostikum/Producto sani-
tario para diagnóstico in vitro/
Dispositivo medico-diagnostico in vitro/
Dispositif médical de diagnostic in vitro/
Medisch hulpmiddel voor in-vitro
diagnostiek/Medicinsk udstyr til in
vitro-diagnostik/In Vitro diagnostický
zdravotnický prostředek /
In Vitro Διαγνωστικό Ιατροτεχνολογικό
πρωτόν/Dispositivo médico para
diagnóstico in vitro/In vitro
diagnostikum/Endast för in vitro-
diagnostik/Wyrób do diagnostyki In
Vitro/In Vitro Diagnostinė Medicinos
Priemonė/Только для диагностики
In Vitro



Temperature limitation/
Temperaturbegrenzung/
Limite de temperatura/
Limiti di temperatura/
Limites de température/
Temperatuurlimiet/
Temperaturbegrænsning/
Teplotni rozmezi od do/
Περιορισμοί θερμοκρασίας/
Limites de temperatura/
Hőmérséklettartomány/
Temperaturbegrænsning/
Przestrzegać zakresu temperatury/
Temperatūriņai apribojimai/
Температурный режим



Consult Instructions for Use/
Gebrauchsanweisung beachten/
Consulte las instrucciones de uso/
Consultare le istruzioni per l'uso/
Consulter les instructions d'utilisation/
Raadpleeg de gebruiksaanwijzing/
Se brugsanvisning/Viz návod k
použití/ Συμβουλευτείτε τις οδηγίες
χρήσης/Consulte as instruções de
utilização/Néze meg a Használati
utasítást/Se bruksanvisning/Sprawdź
w instrukcji obsługi/Dėl naudojimo
žiūrėkite instrukcijas/
Обратитесь к инструкции по
применению



Biological risks/Biogefährdung/
Riesgo biológico/Rischio biologico/
Risques biologiques/Biologisch
risico/Biologisk fare/
Biologicky nebezpečné
Βιολογικοί κίνδυνοι/Risco biológico
Biológiai kockázat/Biologisk risk/
Ryzyko biologiczne/Biologinis pavojus/
Биологическая опасность

ORIG MOU

From mouse/der Maus/de ratón/
Murino/De souris/Mus/απο ποντίκι/
Från mus/Pelès kilmès/
Мышиного происхождения

ORIG HUM

Human/Human/Humano/
Origine Umana/Humaine/Human
δείγματα αναφοράς/Human/
Žmogaus kilmės/
Человеческого происхождения

CONT

Contents of kit/Inhalt/Contenido/
Contenido/Contenu/Indhold/
ανιδραστήρια/Kit innehåll/
Rinkinio turinys/
Компоненты набора

WARNINGS AND PRECAUTIONS

GB

For in vitro diagnostic use

- For Professional Use Only
- Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.
- Handle all patient 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 and 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.

WARNHINWEISE UND VORSICHTSMASSNAHMEN

DE

Für In-vitro-Diagnostik

- Nur für geschultes Fachpersonal.
- Bitte beachten Sie die Vorschriften zur Laborsicherheit in der Publikation Nr. (CDC) 88-8395 des US Department of Health and Human Services (Bethesda, MD, USA) oder andere gleichwertige regionale oder nationale Bestimmungen.
- Alle Patientenproben gelten als potenziell infektiös und sind entsprechend zu handhaben.
- Befolgen Sie die lokalen Richtlinien zur Entsorgung von anfallenden Abfallstoffen.

Achtung

Das zur Herstellung der Reagenzien aus humaner Quelle verwendete Material wurde auf HIV-1/2-Antikörper, HCV-Antikörper und Hepatitis-B-Oberflächenantigen (HBsAg) getestet und als nicht reaktiv befunden. Da es keine Methode gibt, mit der das Vorliegen von durch Blut übertragenen Krankheiten vollkommen ausgeschlossen werden kann, sollten der Umgang mit Reagenzien aus humaner Quelle und deren Entsorgung so erfolgen, als handele es sich um potenziell infektiöses Material.

CUIDADOS Y PRECAUCIONES

ES

Para diagnóstico in vitro

- Solo para uso profesional
- Consultar la publicación del U.S. Department of Health and Human Services (Bethesda, Md., USA) publicación No. (CDC) 88-8395 o las normas locales o nacionales.
- Tratar todas las muestras de pacientes como potencialmente infecciosas.
- Todos los residuos se deben tirar cumpliendo las normas en vigor.

Precaución

Material usado en la preparación de este reactivo se analizó la presencia de anticuerpos HIV 1 y 2, anticuerpos HCV y antígenos de superficie de hepatitis B, siendo el resultado de dichos análisis negativo. Sin embargo, como el test no puede excluir completamente los anticuerpos HIV 1 y 2, anticuerpos HCV y antígenos de superficie de hepatitis B, el manejo y disposición del reactivo debe ser como potencialmente infecciosas.

AVVERTENZE E PRECAUZIONI

IT

Per uso diagnostico in vitro

- Solamente per uso professionale
- Come riferimento si consiglia la pubblicazione No. (CDC) 88-8395 del US Department of Health and Human Service o qualsiasi altro regolamento locale o nazionale relativo alle Norme di Sicurezza da seguire nei Laboratori Diagnostici
- Maneggiare i campioni dei pazienti come potenzialmente infetti
- Seguire le normative vigenti relative all'eliminazione del materiale usato

Precauzioni

Le sostanze usate nella preparazione dei reagenti sono state testate e trovate Non Reattive per l'anticorpo anti-HIV 1 e 2, per l'anticorpo anti-HCV e l'antigene di superficie dell'Epatite B (HbsAg). Tuttavia poiché nessun metodo diagnostico è in grado di escludere completamente la possibilità di trasmissione di infezioni attraverso il sangue si consiglia di maneggiare questi reattivi come potenzialmente infettivi.

PRÉCAUTIONS D'EMPLOI ET MISE EN GARDE

FR

Pour un usage diagnostic in Vitro

- Pour usage professionnel seulement.
- Prière de se référer à la Publication N° : (CDC) 88-8395 de l'U.S. Département of Health and Human Services (Bethesda, Md., USA) sur les procédures de sécurité dans les laboratoires ou toutes autres réglementations locales et nationales.
- Manipuler les échantillons de patients comme potentiellement infectieux.
- Suivre les réglementations locales pour l'élimination et le traitement de tous les déchets.

Attention

Le matériel utilisé pour la préparation de réactifs d'origine humaine, a été testé et trouvé non réactif aux Anticorps anti-virus de l'immunodéficience humaine (VIH-1/2), aux Anticorps de l'Hépatite C (VHC) et à l'Antigène de surface de l'Hépatite B (AgHBs). Puisqu'il n'existe pas de méthode de test, rejetant complètement la présence de maladies dans le sang, la manipulation et l'élimination de réactifs d'origine humaine doivent être effectuées comme s'ils étaient potentiellement infectieux.

ADVARSLER OG FORHOLDSREGLER

DK

Til *in vitro* diagnostisk anvendelse

- Kun til professionel brug
- Der henvises til U.S. Department of Health and Human Services (de amerikanske sundhedsmyndigheder) (Bethesda, Md., USA) udgivelse nr. (CDC) 88-8395 vedrørende laboratoriesikkerhedsprocedurer eller andre lokale eller nationale forskrifter.
- Alle patientprøver skal behandles som potentielt smittefarlige.
- Følg lokale regler for afskaffelse af alt affald.

Advarsel

Alt materiale anvendt ved beredningen af reagenser af human oprindelse er blevet testet og fundet negative for HIV 1 og 2 antistoffer, HCV antistoffer og Hepatitis B overflade antigen (HbsAg). Da ingen analysemetoder fuldstændig kan udelukke tilstedeværelsen af blodbårne sygdomme, skal håndtering og bortskaffelse af reagenser af human oprindelse fra dette produkt behandles som potentielt smittefarligt.

ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ ΚΑΙ ΠΡΟΦΥΛΑΞΕΙΣ

GR

Για *in vitro* διαγνωσική χρήση

- Για επαγγελματική χρήση, μόνο.
- Παρακαλούμαι όπως επικαλεστείτε τις οδηγίες ασφαλούς λειτουργίας των εργαστηρίων του Τμήματος Υγείας και Ανθρώπινων Υπηρεσιών των Η.Π.Α.(U.S. Department of Health and Human Services) (Bethesda, Md., USA) αριθμός έκδοσης (CDC) 88—8395, ή οποιοδήποτε άλλο κατά τόπους σχετικό Εθνικό κανονισμό.
- Μεταχειριστήτε όλα τα δείγματα ως μολυσμένα.
- Ακολουθείστε τις κατά τόπου οδηγίες για απομάκρυνση άχρηστου υλικού.

Προσοχή

Όλα τα υλικά που χρησιμοποιούνται για την παρασκευή αντιδραστηρίων ανθρώπινης προέλευσης έχουν εξετασθεί και έχουν βρεθεί αρνητικά για HIV-1/2 Αντίσωμα (Ab), HCV Αντίσωμα (Ab) και Ηπατίτιδος Β Αντιγόνο Επιφανείας (Hepatitis B Surface Antigen) (HBSAg). Εφόσον δεν υπάρχει μέθοδος ικανή να αποκλείσει απόλυτα την παρουσία αιματολογικών / μολυσματικών ασθενειών, ο τρόπος μεταχείρισης και η απομάκρυνση αντιδραστηρίων ανθρώπινης προέλευσης αυτού του συγκεκριμένου προϊόντος, πρέπει να είναι ίδιος με αυτόν που ακολουθείται για μολυσμένα δείγματα.

VARNINGAR OCH SÄKERHETSÅTGÄRDER

SE

Endast för *in vitro* diagnostik

- Endast för professionellt bruk
- Följ "U.S. Department of Health and Human Services (Bethesda, Md., USA) publikation (CDC) 88-8395" eller annan lokal eller nationell bestämmelse beträffande laboratoriesäkerhet.
- Hantera alla patientprover som potentiellt smittsamma.
- Följ lokala bestämmelser för bortskaffande av avfall.

Varning

Material som använts för tillverkning av reagens med humant ursprung har testats och befunnits negativt för HIV 1 och 2 antikroppar, HCV antikroppar samt hepatit B ytantigen (HBSAg). Eftersom inget test fullständigt kan utesluta ev. närvaro av blodsmitta skall hantering och bortskaffande av humant material från denna produkt ske som om den vore potentiellt infektiös.

CanAg NSE EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg NSE EIA kit is intended for the quantitative determination of NSE in human serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) exists as several dimeric isoenzymes ($\alpha\alpha$, $\alpha\beta$, $\alpha\gamma$, $\beta\beta$ and $\gamma\gamma$) composed of three distinct subunits α , β and γ . The γ unit is found either in a homologous $\gamma\gamma$ - or in a heterologous $\alpha\gamma$ -isoenzyme and is known as neuron-specific enolase (NSE). The monoclonal antibodies used in the CanAg NSE EIA bind to the γ -subunit of the enzyme and thereby detects both the $\gamma\gamma$ and the $\alpha\gamma$ forms (1, 2). The NSE levels are low in healthy subjects and subjects with benign diseases. Elevated levels are commonly found in patients with malignant tumours with neuroendocrine differentiation, especially small cell lung cancer (SCLC) (3) and neuroblastoma (4). Quantitative determination of NSE in serum may be valuable in the management of patients with suspected or diagnosed SCLC or neuroblastoma, to aid in the differential diagnosis and to monitor the effect of treatment (5, 6).

PRINCIPLE OF THE TEST

The CanAg NSE EIA is a solid phase, non-competitive immunoassay based on two monoclonal antibodies (derived from mice) directed against two separate antigenic determinants of the NSE molecule. The monoclonal antibodies (MAb) used bind to the γ -subunit of the enzyme and thereby detects both the $\gamma\gamma$ and the $\alpha\gamma$ form. Calibrators and patient samples are incubated together with biotinylated Anti-NSE MAb E21 and horseradish peroxidase (HRP) labelled Anti-NSE MAb E17 in streptavidin coated micro strips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine) 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 development is proportional to the amount of NSE 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 NSE concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg NSE 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 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminium pouch containing desiccant and reseal carefully to keep dry.

NSE Calibrators	5 vials, lyophilised	4 weeks at 2–8°C 3 months at –20°C
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CAL	NSE	A	1 x 0.75 mL
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CAL	NSE	B	1 x 0.75 mL
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CAL	NSE	C	1 x 0.75 mL
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CAL	NSE	D	1 x 0.75 mL
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CAL	NSE	E	1 x 0.75 mL
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The lyophilised calibrators contain human NSE in a protein matrix with 0.01 % of a non-azide preservative. To be reconstituted with 0.75 mL distilled water before use.

NOTE: The exact NSE concentration is lot specific and is indicated on the label of each vial.

Component	Quantity	Storage and stability after first opening
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BIOTIN	Anti-NSE
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Biotin Anti-NSE	1 x 15 mL	2–8°C until expiry date stated on the vial
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Biotin Anti-NSE monoclonal antibody from mouse, approximately 2 µg/mL. Contains phosphate buffer (pH 7.1), bovine serum albumin, blocking agents, an inert blue dye and 0.01 % methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-NSE before use.

CONJ	Anti-NSE
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Tracer, HRP Anti-NSE	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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Stock solution of HRP Anti-NSE monoclonal antibody from mouse, approximately 40 µg/mL. To be mixed with Biotin Anti-NSE prior to use. Contains 0.02 % methyl-isothiazolone (MIT), 0.02 % bromonitrodioxane and 20 ppm Proclin™ 300 as preservatives.

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

Component	Quantity	Storage and stability after first opening
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STOP

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

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

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

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- Handle all patient specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

Caution

Each donor unit 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 NSE EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Serum should be separated from the clot within 60 minutes of collection to avoid leaking of NSE from blood cells. Do not use haemolysed samples. Plasma is not recommended since significant amounts of NSE can be released from platelets. Samples can be stored at 2–8°C for 24 hours. For longer periods store samples at -70°C or below. Samples should not be stored in a self-defrosting freezer and not be thawed and refrozen before analysis. Bring frozen samples to room temperature and mix THOROUGHLY by gently inverting multiple times before analysis. Samples that contain gross particulates should be centrifuged at 10.000 x g for 10 minutes, prior to use to eliminate any particulate matter that may have developed from the thawing process. Analyze thawed samples within one hour.

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 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 microplate wash 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 for dispensing microlitre volumes. An 8-channel pipette or respenser pipette with disposable plastic tips for delivery of 100 µL is useful but not essential. Pipettes for dispensing millilitre volumes.

5. Distilled or deionized water

For reconstitution of NSE Calibrators and for preparation of diluted wash solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg NSE 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 sera must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators and patient 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 NSE EIA REF 420-10

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

Step	Bottle/Plate	Procedure																		
1. Prepare NSE Calibrators	<table border="1" style="display: inline-table;"> <tr> <td style="padding: 2px;">CAL</td> <td style="padding: 2px;">NSE</td> </tr> </table> A, B, C, D, E	CAL	NSE	Add 0.75 mL of distilled water to each vial and mix gently. Allow to stand for at least 15 minutes. NOTE: The exact concentration of each calibrator is stated on the label. This value of the calibrators should be used for calculations.																
CAL	NSE																			
2. Prepare Wash Solution	<table border="1" style="display: inline-table;"> <tr> <td style="padding: 2px;">WASHBUF</td> <td style="padding: 2px;">25X</td> </tr> </table>	WASHBUF	25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.																
WASHBUF	25X																			
3. Prepare Antibody Solution	<table border="1" style="display: inline-table;"> <tr> <td style="padding: 2px;">CONJ</td> <td style="padding: 2px;">Anti-NSE</td> </tr> <tr> <td style="padding: 2px;">BIOTIN</td> <td style="padding: 2px;">Anti-NSE</td> </tr> </table>	CONJ	Anti-NSE	BIOTIN	Anti-NSE	Mix 50 μ L of Tracer, HRP Anti-NSE with 1 mL of Biotin Anti-NSE per strip:														
CONJ	Anti-NSE																			
BIOTIN	Anti-NSE																			
		<table border="1" style="width: 100%;"> <thead> <tr> <th style="text-align: left;">No. of Strips</th> <th style="text-align: center;">HRP Anti-NSE (μL)</th> <th style="text-align: center;">Biotin Anti-NSE (mL)</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">1</td> <td style="text-align: center;">50</td> <td style="text-align: center;">1</td> </tr> <tr> <td style="text-align: center;">2</td> <td style="text-align: center;">100</td> <td style="text-align: center;">2</td> </tr> <tr> <td style="text-align: center;">3</td> <td style="text-align: center;">150</td> <td style="text-align: center;">3</td> </tr> <tr> <td style="text-align: center;">4</td> <td style="text-align: center;">200</td> <td style="text-align: center;">4</td> </tr> <tr> <td style="text-align: center;">5</td> <td style="text-align: center;">250</td> <td style="text-align: center;">5</td> </tr> </tbody> </table>	No. of Strips	HRP Anti-NSE (μ L)	Biotin Anti-NSE (mL)	1	50	1	2	100	2	3	150	3	4	200	4	5	250	5
No. of Strips	HRP Anti-NSE (μ L)	Biotin Anti-NSE (mL)																		
1	50	1																		
2	100	2																		
3	150	3																		
4	200	4																		
5	250	5																		

5	250
6	300
7	350
8	400
9	450
10	500
11	550
12	600

4. Wash	MICROPLA	Wash each well once with Wash Solution
5. Add calibrators and samples A, B, C, D, E	CAL NSE	25 µL in each well
6. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well
7. Incubate	MICROPLA	1 hour shaking at room temperature
8. Wash	MICROPLA	Wash each well six times with Wash Solution
9. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well
10. Incubate	MICROPLA	30 min shaking at room temperature
11. Read absorbance	MICROPLA	620 nm
Alt.11 Add Stop Solution	STOP	100 µL in each well
Alt.12 Incubate	MICROPLA	1 min shaking at room temperature
Alt.13 Read absorbance	MICROPLA	Read at 405 nm within 15 min

Preparation of reagents	Stability of prepared reagent
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NSE Calibrators

4 weeks at 2–8°C
3 months at -20°C

Add exactly 0.75 mL of distilled water to each vial and mix gently. Allow standing for at least 15 minutes to reconstitute. **NOTE:** The concentration of the calibrators is stated on the labels and should be used for calculation of the results.

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 deionised 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-NSE with 1 mL of Biotin Anti-NSE per strip (see table below):

No. of Strips	Tracer, HRP Anti-NSE (µL)	Biotin Anti-NSE (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 Antibody Solution.

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

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 both calibrators and patient 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 NSE Calibrators, 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 μ L of the NSE Calibrators (CAL A, B, C, D, E) and patient specimens (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	4th Unk				
B	Cal A	Cal E	etc				
C	Cal B	1st Unk					
D	Cal B	1st Unk					
E	Cal C	2nd Unk					
F	Cal C	2nd Unk					
G	Cal D	3rd Unk					
H	Cal D	3rd Unk					

4. Add 100 μ L of Antibody Solution to each well using a 100 μ L precision pipette (or an 8-channel 100 μ 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 surface of the liquid.
5. Incubate the plate for 1 hour (\pm 10 min) at room temperature (20-25°C) with constant shaking of the plate using a microplate shaker.
6. After the incubation aspirate and wash each strip 6 times.

7. Add 100 μ L of TMB HRP-Substrate to each well using the same procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between 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 exposure to 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 in item 10.

10. Add 100 μ L of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg NSE EIA measures concentrations between 1 and approximately 150 μ g/L. If NSE 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 should also be measured in order to determine the endogenous NSE concentration (see "Calculation of results").

Quality control

CanChek Tumor Marker Control Sera Levels 1 and 2 (available separately, REF 107-20) are recommended for validation of the assay series. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated.

Reference materials

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

CALCULATION OF RESULTS

If a microplate spectrophotometer with built-in data calculation program is used refer to the manual for the spectrophotometer and create a program using the concentration stated on the label of each of the NSE calibrators.

For automatic calculation of NSE results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator A should be included in the curve with the value 0 µg/L.
- Spline smoothed curve fit method. Calibrator A should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator A should be included in the curve with the value 0 µg/L.
- Quadratic curve fit method. Calibrator A should be included in the curve with the value 0 µg/L.

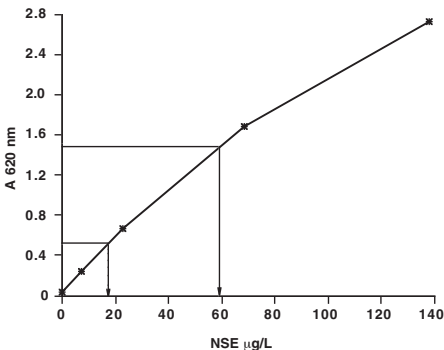
NOTE: 4-Parametric or Linear regression evaluation methods should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each NSE Calibrator against the corresponding NSE concentration (in µg/L), see figure. The unknown NSE concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen. If samples in an initial analysis give NSE levels above the concentration of calibrator E, it is necessary to dilute the sample 1/10 with normal human serum in order to obtain accurate results. The result should then be calculated according to the following procedure:

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

Example of results

Specimen	Calibrator values	Mean abs value (A)	NSE µg/L
CAL NSE A	0 µg/L	0.037	
CAL NSE B	7.5 µg/L	0.238	
CAL NSE C	22.9 µg/L	0.663	
CAL NSE D	68.4 µg/L	1.688	
CAL NSE E	138.0 µg/L	2.720	
Specimen 1		0.518	17.5
Specimen 2		1.474	57.8



Example, do not use this curve to determine assay results.

The exact NSE concentration is indicated on the label of each calibrator vial.

LIMITATIONS OF THE PROCEDURE

The level of NSE cannot be used as absolute evidence for the presence or absence of malignant disease and the NSE test should not be used in cancer screening. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the NSE test should not replace any established clinical examination.

Elevated NSE values not due to tumours may occur in dialysis patients and patients with leukaemic diseases.

Serum should not contain visible haemolysis (the absorbance at 500 nm for non-turbid sample should not exceed 0.3) since erythrocytes contain significant amounts of NSE (7). Prolonged storage of whole blood can cause release of NSE from the blood cells.

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

EXPECTED VALUES

Healthy individuals are expected to have NSE values below 13 µg/L. It is recommended that each laboratory establish its own normal range to account for such local environmental factors as diet, climate, living conditions, patient selection, etc.

PERFORMANCE CHARACTERISTICS

Precision

Total precision was determined according to NCCLS guideline EP5-A (8) using four levels of frozen pooled human serum containing added NSE. Each sample was randomly pipetted in duplicates and analysed twice each day over 20 days. The analyses were undertaken during a period of 40 months, by \geq three different technicians and using 20 different CanAg NSE EIA kit batches.

Sample	Replicates	Mean $\mu\text{g/L}$	Within-run SD ($\mu\text{g/L}$)	Within-run CV %	Between-day SD ($\mu\text{g/L}$)	Between-day CV %
NSE 1	80	10.3	0.24	2.3	0.57	5.5
NSE 2	80	23.7	0.82	3.5	0.97	4.1
NSE 3	80	48.2	1.02	2.1	1.93	4.0
NSE 4	80	92.7	1.60	1.7	3.44	3.7

Detection limit

The detection limit of the CanAg NSE EIA assay is $< 1 \mu\text{g/L}$ defined as the concentration corresponding to the mean of the absorbance values for the NSE Calibrator A plus 2 standard deviations according to the formula:

$$\frac{2 \times \text{SD CAL A}}{\text{OD CAL B} - \text{OD CAL A}} \times [\text{CAL B}] \mu\text{g/L}$$

Hook effect

No hook effect has been noticed for NSE concentrations up to 200 000 $\mu\text{g/L}$.

Linearity

Patient samples were diluted with normal serum and analysed. The obtained values were in the range 93–101 % of the expected values.

Specificity

The monoclonal antibodies used are specific for the γ -subunit of enolase. No measurable cross-reactions with other enolase have been observed.

The NCCLS guideline EP7-P (9) was followed to determine possible sources of interference. The following substances and concentrations were tested and found not to interfere with the test.

	Concentration with no significant (\pm 10%) interference
Lipemia (Intralipid®)	10 mg/mL
Bilirubin, unconjugated	0.6 mg/mL

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.

LITERATURE REFERENCES

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