CanAg NSE EIA

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

REF **420-85**

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/Utilizare 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

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/Dijagnostički 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

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/ Katalogusnummer/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/

Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Indeholder tilstrækkeligt/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/ Prečitajte 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/С 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/С миши произход/Myší/ Fra mus/Maus/απο 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/С 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/Hovädzie/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/Rozriedťe 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 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).

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 samples are incubated together with biotinylated Anti-NSE MAb E21 and horseradish peroxidase (HRP) labelled Anti-NSE MAb E17 in streptavidin coated microstrips. 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 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
MICROPLA		
Microplate	1 Plate	2–8° C until expiry date stated on the plate
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
CAL NSE A	1 x 0.75 mL	
CAL NSE B	1 x 0.75 mL	
CAL NSE C	1 x 0.75 mL	
CAL NSE D	1 x 0.75 mL	
CAL NSE E	1 x 0.75 mL	

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

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

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

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

STOP		
Stop Solution	1 x 15 mL	2–8° C until expiry date stated on the vial

Ready for use. Contains 0.12 M hydrochloric acid.

Component	Quantity	Storage and stability after first opening
WASHBUF 25X		
Wash Concentrate	1 x 50 mL	2–8° C until expiry date stated on the bottle

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

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

- Please refer to the U.S. 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

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.

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 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, 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 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 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 420-85

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

Step	Vial/Plate	Procedure
1. Prepare NSE Calibrators	CAL | NSE A, B, C, D, E	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.
2. Prepare Wash Solution	WASHBUF | 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.
3. Prepare Antibody Solution	CONJ | Anti-NSE BIOTIN | Anti-NSE	Mix 50 µL of Tracer, HRP Anti-NSE with 1 mL of Biotin Anti-NSE per strip:

No. of Strips	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
4.	Wash	MICROPLA	Wash each well once with Wash Solution
5.	Add calibrators and samples	CAL | NSE A, B, C, D, E	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 5 min

Preparation of reagents	Stability of prepared reagent
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 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 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 µL of the NSE Calibrators (CAL A, B, C, D, E) and unknown 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 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 surface of the liquid.
5. Incubate the plate for 1 hour (± 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 µL 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 (± 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 µL of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 5 min after addition of Stop Solution.

Measurement range

The CanAg NSE EIA measures concentrations between 1 and approximately 150 µg/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").

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.

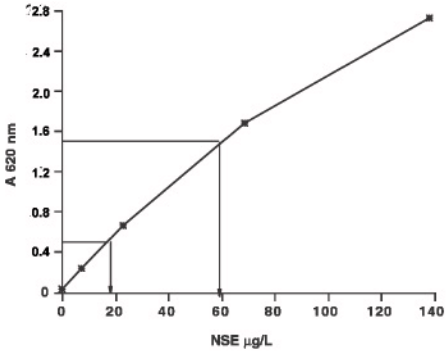
NOTE: 4-Parametric or Linear regression evaluation methods should not be used.

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 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:

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

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

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 (3). 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 sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer.

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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