

NSE [¹²⁵I] IRMA KIT

(REF: RK-300CT)

The NSE IRMA system provides a direct *in vitro* quantitative determination of Neuron Specific Enolase in human serum in the range of 0-200 ng/mL. Each kit contains material sufficient for 100 assay tubes, permitting the construction of one standard curve and the assay of 42 unknowns in duplicates.

Introduction

Neuron Specific Enolase is a glycolytic enzyme normally present in neurons, central and peripheral nervous tissues and neuroendocrine cells. It exists in the form of dimers $\alpha\gamma$ and $\gamma\gamma$ with a molecular weight of approximately 95 kDa.

Elevated serum concentrations of Neuron Specific Enolase can be found in patients with malignant tumors of neuroectodermic or neuroendocrine origin, for example neuroblastoma, small-cell lung carcinoma (SCLC), pheochromocytoma and medullar thyroid cancer.

The NSE assay is not useful as a screening test. However, it is of aid in monitoring disease progression, treatment efficiency, and in the early detection of relapse and long-term follow up of patients.

Principle of method

The technology uses two monoclonal antibodies of high affinity in an immunoradiometric assay (IRMA) system. The ¹²⁵I labelled signal-antibody binds to an epitope of the molecule spatially different from that recognized by the biotin-capture-antibody. These antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During an incubation period of 1 hour with shaking, the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. The reaction mixture is then discarded, the test tubes are washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve, plotting binding values against a series of calibrators containing known amounts of NSE, the unknown concentration of patient samples can be determined.

Contents of the kit

- One bottle of TRACER (21 mL), ready to use, containing < 980 kBq ¹²⁵I-anti- NSE antibody and biotin-capture antibody in buffer with red dye and 0.1 % NaN₃.
- One vial of STANDARD ZERO S0 (2.5 mL), ready to use, bovine serum with 0.1% NaN₃.
- Five vials of STANDARDS S1-S5 (5 x 0.5 mL), freeze-dried, in bovine serum with 0.1% NaN₃. The concentrations of

standards are specified in the quality certificate enclosed.

- Two vials of CONTROL SERA (2x0.5 mL), freeze-dried, in human serum with 0.1% NaN₃. The concentrations of controls are specified in the quality certificate enclosed.
- COATED TUBES, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
- One bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN₃. See *Preparation of reagents*.

Quality certificate

Pack leaflet

Materials, tools and equipment required

- common laboratory equipment
- 50 μ L precision micropipette
- 200 μ L repeating pipette
- 2000 μ L repeating pipette or dispenser
- horizontal shaker (at least 600 rpm)
- plastic foil to cover tubes
- absorbent tissue
- gamma-counter with software

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice.

Erythrocytes and thrombocytes can release significant amounts of NSE into serum. The separation from red cells must occur within 60 minutes after the venipuncture. Serum should not contain visible hemolysis.

Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Before assaying, frozen samples should be thawed and gently mixed by inversion.

Samples with a NSE concentration higher than 200 ng/mL should be diluted twice (1:1) with S0 zero standard and reassayed.

Preparation of reagents, storage

Store the reagents between 2-8°C. At this temperature each reagent is stable until the expiration date of the kit.

Reconstitute Standards and Controls with 0.5 mL distilled water before use. After 20 minutes, mix by inversion or vortex gently. ***For further use, reconstituted components have to be stored at -20°C until the expiry date of the kit.***

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. After dilution, store at 2-8°C until the expiration date of the kit.

Assay procedure

(For a quick guide, refer to Table 1.)

- Label coated tubes in duplicate for each standard (S0-S5), control serum (CI, CII) and samples. Label two test tubes for total counts (T).
- Pipette 50 μ L of standards, controls and samples into the properly labeled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
- Pipette 200 μ L of tracer into each tube. (except T)
- Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm).
- Incubate tubes for 1 hour, shaking at room temperature.
- Add 2.0 mL diluted wash buffer to each tube. Decant the content of tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
- Return the tube-rack to an upright position and repeat step-6 two more times.
- Count each tube for at least 60 seconds in a gamma counter.
- Calculate the NSE concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		50		
Control			50	
Sample				50
Tracer	200	200	200	200
Shake for 1 hour at room temperature				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2. Calculate the average count per minute (CPM) for each pair of assay tubes. Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1-5} / C_{I-II} / M_x \text{ (cpm)} - S_0 \text{ (cpm)}}{T \text{ (cpm)}} \times 100$$

Using semi-logarithmic graph paper plot the B/T(%) for each standard versus the corresponding concentration of NSE. Determine the NSE concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range. Out of fitting programs applied for computerized data processing, spline fittings are recommended.

Table 2. Typical assay data

Tubes	Mean cpm	B/T%	NSE ng/mL
T	405199		
S0	202	0.05	0
S1	1570	0.34	1.8
S2	4028	0.95	5.6
S3	11947	2.90	20
S4	33007	8.10	60
S5	88334	21.75	200
CI	6045	1.44	9.0
CII	15041	3.66	25.0

Performance characteristics

Specificity

The antibodies used in this assay are specific for the α subunit of NSE. Cross-reactivity to NNE (non-neuronal enolase) is < 1.6%.

Sensitivity

Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:

Limit of Blank (LoB): 0.03 ng/mL

Limit of Detection (LoD): 0.22 ng/mL

For results under LoB, should report as "analyte not detected". For results between LoB and LoD, should report as "analyte detected", concentration < 0.22 ng/mL.

Precision and reproducibility

Four serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 20 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (ng/mL)	CV%	Mean (ng/mL)	CV%
7.55	2.39	7.74	4.90
16.59	1.96	17.34	3.29
35.23	1.24	36.78	4.81
96.85	1.74	95.92	3.48

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of NSE. The average per cent recovery for 5 serum samples spiked with NSE at 3 levels each was 108%, with a range of 103% to 114%.

Hook effect

No hook effect is observed for concentrations lower than 20000 ng/mL.

Expected Values

It is recommended that each laboratory determine a reference range for its own patient population.

Serum samples from 245 presumably healthy blood donors were evaluated.

Samples	245
Mean (ng/mL)	7.25
Median (ng/mL)	7.09
Samples < 12 ng/mL	241 (98.4%)

Procedural notes

- The non-respect of the instructions in this insert may affect results significantly.
- Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
- Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

Limitations

- The NSE assay should not be used as a cancer screening test.
- A NSE value below 12 ng/mL does not indicate the absence of residual cancer.
- Values higher than 12 ng/mL may occur in dialysis patients.
- Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other diagnostic procedures.
- Specimens from patients who have received mouse immunoglobulin for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Serum from such individuals may produce erroneous results.

Precautions

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen

(HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*. All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 67 mg.

	Use by	CONTROL	Control
	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
	In vitro diagnostic medical device		Store between 2-8°C
	Manufacturer		Radioactive material
REF	Catalogue number		

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