Datasheet for ABIN1305165

ENO2/NSE ELISA Kit

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Overview

Quantity:	96 tests
Target:	ENO2/NSE (ENO2)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.2-178 ng/mL
Minimum Detection Limit:	1.2 ng/mL
Application:	ELISA

Product Details

Purpose:	This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human neuron specific enolase (NSE) levels in patient serum samples. The test might be used as an aid for detecting patients with neuronendocrine differentiated tumors such as small cell lung cancer and neuroblastoma, melanoma, seminoma, and with injury of central nervous system such as traumatic brain injury (TBI).
Brand:	ED™
Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. Streptavidin Coated Microplate Two sets of two vials each containing human NSE in a lyophilized bovine serum based matrix with a non-azide, non-mercury based preservative. Refer to vials for exact concentration range

Product Details

	for each control. Both controls should be stored at 2-8 °C and are stable until the expiration date on the kit box.
Material not included:	1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL etc. 2. Repeating dispenser suitable for delivering 100 µL. 3. Disposable pipette tips suitable for above volume dispensing. 4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes. 5. Disposable plastic 100 mL and 1000 mL bottle with caps. 6. Aluminum foil. 7. Deionized or distilled water. 8. Plastic microtiter well cover or polyethylene film. 9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system. 10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Target Details

Target:	ENO2/NSE (ENO2)
Alternative Name:	Neuron Specific Enolase (NSE) (ENO2 Products)
Background:	The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolyase) exists as several dimeric isoenzymes (alpha alpha, alpha beta, beta beta and gamma gamma) composed of three distinct subunits: alpha, beta, and gamma. Three isoenzymes are found in human brain: alpha alpha, alpha beta, and gamma gamma. The heterologous alpha gamma-isoenzyme and the homologous gamma gamma-enolase isoenzymes are known as neuron-specific enolase (NSE) as these isoenzymes initially were detected in neurons and neuroendocrine cells. This test detects both the alpha gamma and the gamma gamma forms by using monoclonal antibodies specific to the gamma-subunit of the enzyme. NSE levels are quite low in normal healthy people and in people with benign disease. Lung cancer is one of the most common cancer forms with incidences about 50-100 per 100,000 population. Approximately 20 % of the lung cancer is small cell lung cancer. NSE has been shown to be a valuable tumor marker of neuroendocrine origin, particularly in small cell lung cancer and in neuroblastoma. Although NSE is similar to Chromogranin A in detecting small cell lung cancer and neuroblastoma, Chromogranin A seems better in detecting carcinoid.
Molecular Weight:	46 kDa
Gene ID:	2026
NCBI Accession:	NP_001966.1

Target Details

UniProt:	P09404

Application Details

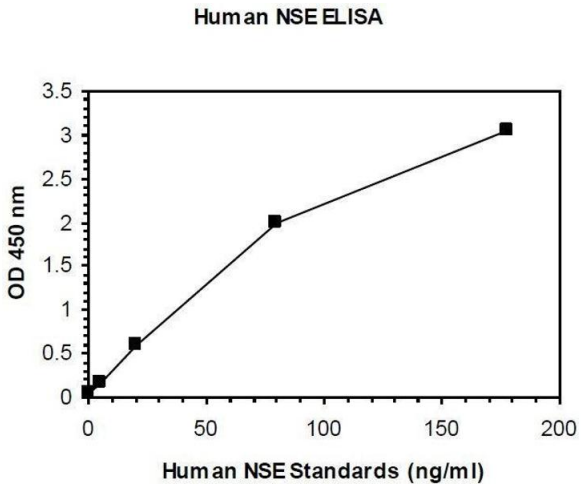
Sample Volume:	20 µL
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	Assay standards, controls and patient samples are added directly to wells of microplate that is coated with a streptavidin. Subsequently, a mixture of a biotinylated NSE specific monoclonal antibody and a horseradish peroxidase (HRP)-labeled NSE specific monoclonal antibody is added to each microtiter well. After the first incubation a sandwich immunocomplex of streptavidin-biotin-monoclonal antibody human NSE monoclonal antibody-HRP is formed. The unbound monoclonal antibodies are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the NSE on the wall of the microtiter well is directly proportional to the amount of NSE in the sample. A standard curve is generated by plotting the absorbance versus the respective human NSE concentration for each standard
Reagent Preparation:	(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged. (2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details. (3) Reconstitute all assay standards and controls by adding 0.5 mL of deminerialized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls should be stored at 2-8 C for up to 30 days. It is not recommended to freeze the reconstituted standards and controls.
Sample Collection:	Only 20 µL of human serum is required for human NSE measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 ? 1500xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 - 8 C if the assay is to be performed within 24 hours. Otherwise, patient samples should be stored at -20 °C or below until

	measurement. Avoid any repeated freezing and thawing of specimen.
Assay Procedure:	(1) Place a sufficient number of streptavidin coated microwell strips in a holder to run human NSE standards, controls and unknown samples in duplicate. (2) Test Configuration (3) Prepare NSE Tracer Antibody and Capture Antibody working solution by 1:21 fold dilution of the Tracer Antibody with the biotinylated Capture Antibody . For each strip, it is required to mix 1 mL of the Capture Antibody with 50 µL of the Tracer Antibody in a clean test tube. (4) Add 10 µL of standards, controls and patient samples into the designated microwell. (5) Add 100 µL of above mixture of Tracer Antibody and Capture Antibody solution to each of the wells. (6) Cover the plate with the plate sealer and incubate plate at room temperature, shaking at 170 rpm for 1 hour. (7) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (8) Add 100 µL of ELISA HRP Substrate into each of the wells (9) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. (10) Incubate plate at room temperature for 10 minutes or less. (11) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (12) Read the absorbance at 450 nm within 10 minutes in a microplate reader. NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm, 620 nm or 630 nm.
Calculation of Results:	1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance. 3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. We recommend using Point-to-Point curve fit.
Assay Precision:	The intra-assay precision is validated by measuring two controls samples in a single assay with 20-replicate determinations. The inter-assay precision is validated by measuring two control samples in duplicate in 12 individual assays.
Restrictions:	For Research Use only

Handling

Precaution of Use:	The reagents must be used in a professional laboratory environment and are for research use only. Source material of bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
Storage:	4 °C

Images



ELISA

Image 1.

Validation report #103295 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

by Vascular Surgery, University Hospital RWTH Aachen

Report Number: 103295

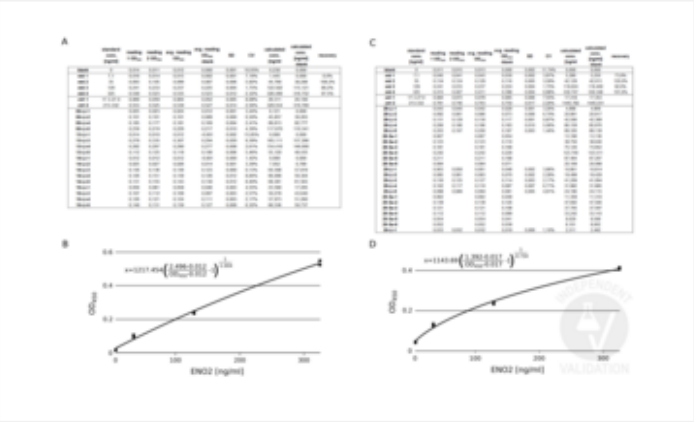
Date: Jun 25 2018

Target:	ENO2
Lot Number:	K591
Method validated:	ELISA (ELISA)
Positive Control:	Serum and liquor samples from seven human patients after surgery
Negative Control:	Blank controls
Spike Control:	Spike controls provided with the kit
Notes:	Passed. The human ENO2 ELISA kit ABIN1305165 specifically measures detects the antigen in human serum and liquor samples.
Standard Curve:	Two sets of five vials each containing human ENO2 at concentrations 0, 7.1, 35, 129, and 325ng/ml
Protocol:	• Reagents were prepared and stored according to the user manual. • Assay instructions were followed, considering preparation for two tests with half a plate each. • Prepare NSE Tracer Antibody and Capture Antibody working solution by 1:21 fold dilution of the Tracer Antibody with the biotinylated Capture Antibody. • Add 10µl of standards, controls and patient samples into the designated microwell. • Add 100µl of above mixture of Tracer Antibody and Capture Antibody solution to each of the wells. • Cover the plate with the plate sealer and incubate plate at RT, shaking at 170rpm for 1h. • Remove plate sealer. Decant the contents of each well. Wash each well 5x by dispensing 350 µl of working wash solution into each well and then decant the contents. • Add 100µL of ELISA HRP Substrate into each of the wells. • Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. • Incubate plate at for at RT for 10min. • Remove the aluminum foil and plate sealer. Add 100µl of ELISA Stop Solution into each of the wells. Mix gently. • Read the absorbance at 450nm within 10min in a microplate reader.
Experimental Notes:	• The ENO2 capture antibody appears still cloudy after mixing tracer and capture antibodies. • We have measured ENO2 in patient serum and liquor samples at different time points post-

surgery. Most of our samples were in the range of the standard curve. We are evaluating ENO2 in context of spinal ischemia and have no prior experience with the marker's concentration subsequently to surgery. The increased ENO2 concentration in response during the course of the surgery and at later time points was consistent with our expectations. Further measurements are underway with samples from more patients.

- Serum samples from two patients could only be measured without replicates.
- The higher concentrated spike control in the second test (see figure C) gave an abnormally high reading, probably due to a pipetting error.

Image for Validation report #103295



Validation image no. 1 for Enolase 2 (Gamma, Neuronal) (ENO2) ELISA Kit (ABIN1305165)

A. Standard curve (std), spike control (ctrl) and serum (Se) and liquor (Li) measurements at different timepoints (1-5) from patients 05, 13, 14, and 15 using ABIN1305165 microwells A1-H6. B. The standard curve for the first assay was fitted with a 4-Parameter Logistic model, giving good quality fits (R^2=0.995). C. Standard curve (std), spike control (ctrl) and serum (Se) and liquor (Li) measurements at different timepoints (1-6) from patients 28, 29 and 30 using ABIN1305165 microwells A7-H12. D. Standard curve for the second assay fitted with a 4-Parameter Logistic model (R^2 =0.995).