

**AccuDiag™  
 NSE  
 ELISA KIT**

**Cat# 6334-16**



<b>Test</b>	<b>Prostate Specific Antigen ELISA</b>
<b>Method</b>	<b>Enzyme Linked Immunosorbent ELISA</b>
<b>Principle</b>	<b>Peroxidase - Conjugated ELISA</b>
<b>Detection Range</b>	<b>0-200 ng/mL</b>
<b>Sample</b>	<b>25 µl serum</b>
<b>Specificity</b>	<b>98.7%</b>
<b>Sensitivity</b>	<b>0.5 ng/ml</b>
<b>Total Time</b>	<b>~ 80 min</b>
<b>Shelf Life</b>	<b>12 - 14 Months from the manufacturing date</b>

**INTENDED USE**

The Neuron-Specific Enolase (NSE) enzyme linked immunosorbent assay (ELISA) provides quantitative measurement of human NSE in serum to aid in the clinical evaluation of patients suspected of having small cell lung cancer, and other related diseases.

**SUMMARY AND EXPLANATION**

The glycolytic enzyme enolase (2-phosph-D-glycerate hydrolyase) exists as several dimeric isoenzymes ( $\alpha\alpha$ ,  $\alpha\beta$ ,  $\alpha\gamma$  and  $\gamma\gamma$ ) composed of three distinct subunits,  $\alpha$ ,  $\beta$ , and  $\gamma$ . Three isoenzymes are found in human brain:  $\alpha\alpha$ ,  $\alpha\gamma$  and  $\gamma\gamma$ . The  $\alpha\gamma$  and  $\gamma\gamma$ -enolase isoenzymes are also known as neuron-specific enolase (NSE) as these isoenzymes initially were detected in neurons and neuroendocrine cells. The NSE levels are low in health and benign subjects. Elevated levels are commonly found in patients with malignant tumors with neuroendocrine differentiation, especially small cell lung cancer and neuroblastoma.

Lung cancer is one of the most spread cancer forms with incidences about 50~100 per 100,000 population. Approximately 20% of the lung cancer is small cell lung cancer. Patients with small cell lung cancer show various proportions of  $\alpha\gamma$  and  $\gamma\gamma$  isoenzyme. The determination of NSE should detect and  $\gamma$  isoforms with the same sensitivity (1). The antibodies for this particular assay are specific for the  $\gamma$ -subunit without cross reactivity with  $\alpha$  or  $\beta$  subunits (1).

NSE Kits are reported to be useful diagnostic marker for lung cancer (2), neuroblastoma (3), and melanoma (4), seminoma (S) and in injury of central nervous system (6). In addition to the above, NSE can be a valuable tool in following-up the effect of chemotherapy of small cell lung cancer, in prognostic evaluation of patients with small cell lung cancer, and in differential diagnosis between cell lung cancer and non-small cell lung cancer.

**TEST PRINCIPLE**

The NSE Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti- $\gamma$ NSE antibody for solid phase (microtiter wells) immobilization and another monoclonal anti- $\gamma$ NSE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the antibody coated microtiter wells. During the incubation, specific NSE bound to anti-NSE antibody on the wells. Unbound NSE antigen is removed by washing the wells with buffer. Enzyme conjugate is then added to each well. After another incubation, unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the NSE present in each sample. Upon addition of the substrate and chromogen, the intensity of blue color will develop in proportion to the concentration of NSE antigen in the samples.

**SPECIMEN COLLECTION AND PREPARATION**

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. For prolong storage of whole blood can cause release of NSE from the blood cells. If sera can not be assayed immediately, they can be stored at 2~8°C for one day or frozen at -20°C for up to 30 days prior to assay. Sample should not be refrozen. Repeated freezing and thawing is not recommended. Do not store in self-defrosting freezer.
4. Do not use hyperlipemic, hemolyzed, Plasma is not recommended since significant amounts of NSE can be released from the thrombocytes..
5. Avoid turbid and contaminated samples.

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits**

1. Monoclonal anti-NSE antibody coated microtiter plate with 96 wells
2. Sample diluent , 12 ml.
3. Enzyme conjugate reagent, 12 ml.
4. Lyophilized NSE reference standards containing; 0, 5, 15, 40, 100, and 200 ng/ml of NSE. 1 set.
5. 50 X Wash Buffer Concentrate, 15 ml.
6. TMB Substrate, 12 ml.
7. Stop Solution 12ml.

**Materials required but not provided:**

1. Precision pipettes and tips, 0.025ml, 0.05 ml, 0.10 ml, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Glass tubes or flasks to prepare wash buffer.
5. Vortex mixer.
6. Absorbent paper or paper towel.
7. Microtiter plate reader.
8. Graph paper.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18-22°C) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. Add 1 ml of distilled water to reconstitute the lyophilized standards. Allow the reconstituted materials to stand for at least 20 minutes. Mix gently. The reconstituted standards should be stored sealed at 2-8°C.

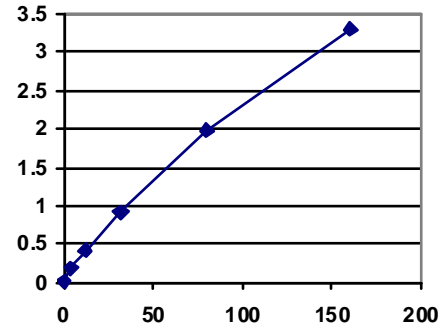


3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750ml of washing buffer (1x). Mix well before use.

NSE Values (ng/ml)	Absorbance (450nm)
0	0.010
5	0.195
15	0.418
40	0.928
100	1.980
200	3.309

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 25µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Sample diluent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100µl TMB substrate into each well. Gentle mix for 5 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100µl of stop solution into each well.
17. Gently mix for 30 seconds to make sure that the blue color changes to yellow color completely.
18. Read optical density at 450nm with a microtiter reader within 30 minutes.



This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.

**Important Note:**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.
4. If a serum specimen contains greater than 200 ng/ml of NSE the sample must be diluted with sample diluent and re-assayed as described in the assay procedure

**EXPECTED VALUES AND SENSITIVITY**

1. It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its environmental factors such as diet, climate etc.
2. A clinical study of the NSE Quantitative kit was conducted and results are summarized as follows: Nearly all the individuals have NSE values below 15 ng/ml (95<sup>th</sup> percentile).
3. The expect ranges are representative only, and do not necessarily reflect the ranges that will be observed in a particular clinical laboratory.

**RESULTS**

Calculate the mean absorbance value for each set of NSE reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of NSE in units per ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

**EXAMPLE OF STANDARD CURVE**

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against NSE concentrations shown in the X axis.

**PERFORMANCE CHARACTERISTICS**

- I. Accuracy:** Serum Comparison between Our Assay kit and a reference method provide the following data

Range From	0.55	~	213.1
N		=	120
Correlation Coefficient		=	0.9909
Slope		=	0.9677
Intercept		=	0.1472
Our Mean		=	21.356
Ref. Mean		=	20.814

## II. Precision.

### 1. Intra-Assay

Concentrations	Replicates	Mean	S.D.	% CV
Level I	20	2.22	0.101	4.54%
Level II	20	6.24	0.287	4.60%
Level III	20	52.17	1.965	3.77%

### 2. Inter-Assay

Concentrations	Replicates	Mean	S.D.	% CV
Level I	10	2.43	0.154	6.35%
Level II	10	6.48	0.330	5.09%
Level III	10	54.04	2.615	4.84%

## III. Linearity

Two patient sera were serially diluted with 0 ng/mL standard in a linearity study. The average recovery was 103.0 %.

Sample A			
Dilution	Expected	Observed	% Recov.
undiluted	102.0	102.0	
2x	51.0	52.2	102.4%
4x	25.50	26.35	103.3%
8x	12.75	14.01	109.9%
16x	6.38	6.12	96.0%
Average Recovery: 102.9 %			

Sample B			
Dilution	Expected	Observed	% Recov.
undiluted	129.3	129.3	
2x	64.7	61.89	95.7%
4x	32.3	31.76	98.3%
8x	16.2	17.45	108.0%
16x	8.1	8.91	110.3%
Average Recovery: 103.1 %			

## IV. Sensitivity

The sensitivity is defined as the concentration of NSE that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The minimum detectable concentration of this assay is estimated to be 0.5 ng/ml

## V. Cross-reactivity

The following cancer marker antigens at high concentrations, as seen in cancer patients, were assayed to determine the possible reactivity.

Antigens	Concentration	% Cross-react.
PAP	500 ng/ml	0
AFP	10,000 ng/mL	0
CEA	5,000 ng/mL	0
CA 125	1,000 U/mL	0
CA 15-3	1,000 U/mL	0
CA 19-9	1,000 U/mL	0
$\alpha$ -HCG	1,000 ng/ml	0
$\beta$ -HCG	1,000 ng/mL	0
HCG	50,000 mIU/ml	0

## VII. Hook Effect

No hook effect was observed in this assay.

## VIII. Hook Effect

Store at 2°C ~ 8°C, the shelf life of the kits will be 12 months

## LIMITATIONS OF PROCEDURE



- For diagnostic purposes, the NSE test results must be used in conjunction with other data available to the physician.
- The NSE test should not be used in cancer screening and should not replace any established clinical examination.
- Samples with NSF level above 200 ng/ml should be diluted to obtain accurate value.
- High NSE values may be found in dialysis patients with leukaemic diseases.
- Serum should not contain visible hemolysis since erythrocytes contain significant amounts of NSE.
- Prolonged storage of whole blood can cause release of NSE from the blood cells.

## STORAGE

- Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
- Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
- A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

## REFERENCES

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<p><b>ISO 13485</b> <b>ISO 9001</b></p>  <p> <b>Diagnostic Automation/ Cortez Diagnostics, Inc.</b>                  23961 Craftsman Road, Suite E/F,                  Calabasas, California 91302 USA</p>			
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<table border="1" style="display: inline-table;"> <tr> <td>EC</td> <td>REP</td> </tr> </table>	EC	REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a>
EC	REP		
Revision B Date: 02-03-2014			