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REF

Cat # 9056-16

CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
NEURON-SPECIFIC ENOLASE
(NSE)

NSE

Cat # 9056-16

CHEMILUMINESCENCE Enzyme Immunoassay for the Quantitative Measurement of NEURON-SPECIFIC ENOLASE (NSE) in Human Serum.

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the

methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL1 and MPL2** microplate luminometers from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF NSE IMMUNOASSAY

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, α , β , and γ . 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 neuronendocrine cells. The NSE levels are low in health and benign subjects. Elevated levels are commonly found in patients with malignant tumors with neuronendocrine 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 γ isoforms with the same sensitivity (1). The antibodies for this particular assay are specific for the γ -subunit without cross reactivity with α or β subunits(1).

NSE are reported to be useful diagnostic marker for lung cancer(2), neuroblastoma(3), 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.

PRINCIPLE OF THE TEST

The NSE Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti- γ NSE antibody for solid phase (microtiter wells) immobilization and another monoclonal anti- γ 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.

A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of NSE antigen in the sample. By reference to a series of NSE standards assayed in the same way, the concentration of NSE in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials Provided with Test Kit

1. Anti-NSE antibody coated microtiter plate , 96 wells.
2. Sample diluent , 12ml
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. 50x Wash Buffer Concentrate, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml

7. Chemiluminescence Reagent B, 6.0 ml

Materials Required but not Provided

1. Distilled water.
2. Precision pipettes: 5–40 μ l, 0.05–0.2ml, 1.0ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

REAGENT PREPARATION

1. Add **0.5 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
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.
3. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense **25 μ l** of standard, specimens, and controls into appropriate wells.
3. Dispense **100 μ l** 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 (18–22°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 **100 μ l** 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 100 μ l Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
15. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

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 180 ng/ml of NSE the sample must be diluted with sample diluent and re-assayed as described in the assay procedure
5. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

CALCULATION OF RESULTS

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against NSE concentration in Units/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of NSE in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

NSE Values (ng/ml)	Relative Light Units (RLU) (10^5)
0	0.02
5	1.10
15	2.64
40	6.02
100	13.20
200	21.21

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 (95th percentile).
3. The expected ranges are representative only, and do not necessarily reflect the ranges that will be observed in a particular clinical laboratory.

Limitations and applications

1. For diagnostic purposes, the NSE test results must be used in conjunction with other data available to the physician.
2. The NSE test should not be used in cancer screening and should not replace any established clinical examination.
3. Samples with NSE level above 180 ng/ml should be diluted to obtain accurate value.
4. High NSE values may be found in dialysis patients with leukaemic diseases.
5. Serum should not contain visible hemolysis since erythrocytes contain significant amounts of NSE.
6. Prolonged storage of whole blood can cause release of NSE from the blood cells.

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