ab217778 – Human Neuron-specific Enolase SimpleStep ELISA® Kit

For the quantitative measurement of Neuron-specific Enolase in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, CSF, and cell and tissue extracts.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab217778

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human Neuron-specific Enolase Capture Antibody 10X	600 µL	+4°C
Human Neuron-specific Enolase Detector Antibody 10X	600 µL	+4°C
Human Neuron-specific Enolase Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Method for determining protein concentration (BCA assay recommended)
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 100 mL 1X Wash Buffer PT combine 10 mL Wash Buffer PT 10X with 90 mL deionized water. Mix thoroughly and gently.

1X Cell Extraction Buffer PTR: Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Cell Extraction Buffer PTR + Enhancer (For cell extraction only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

For serum, plasma, cell culture supernatant, and cerebrospinal fluid samples:

1. Reconstitute the Neuron-specific Enolase standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 40 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	150	150	40,000	20,000
2	Standard#1	150	150	20,000	10,000
3	Standard#2	150	150	10,000	5,000
4	Standard#3	150	150	5,000	2,500
5	Standard#4	150	150	2,500	1,250
6	Standard#5	150	150	1,250	625
7	Standard#6	150	150	625	312.5
8	Blank Control	0	150	0	0

For cell and tissue extract samples:

1. Reconstitute the Neuron-specific Enolase standard sample by adding the volume of 1X Cell Extraction Buffer PTR indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 40 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	150	150	40,000	20,000
2	Standard#1	150	150	20,000	10,000
3	Standard#2	150	150	10,000	5,000
4	Standard#3	150	150	5,000	2,500
5	Standard#4	150	150	2,500	1,250
6	Standard#5	150	150	1,250	625
7	Standard#6	150	150	625	312.5
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	≤50%
Plasma – Citrate	≤50%
Plasma – EDTA	≤50%
Plasma – Heparin	≤50%
Cell Culture Media	≤50%
Cerebrospinal Fluid	≤50%
SH-SY5Y Cell Extract	6.25 µg/mL -100 µg/mL
Brain Cortex Tissue Extract	0.78 µg/mL - 12.5 µg/mL
Hippocampus Tissue Extract	7.81 µg/mL -125 µg/mL

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid (CSF) Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled **1X Cell Extraction Buffer PTR + Enhancer**. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled **1X Cell Extract Buffer PTR+ Enhancer** directly to the plate (use 750 µL - 1.5 mL **1X Cell Extract Buffer PTR + Enhancer** per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.
Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and
store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls, and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

The standard protein in this kit is full-length human Neuron-specific Enolase.

Native signal was detected in cell and tissue extract sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, and CSF.

Urine, saliva, and milk samples have not been tested with this kit.

CROSS REACTIVITY

50 ng/mL of recombinant Human ENO1 was tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE

50 ng/mL of recombinant Human ENO1 was tested for interference with 5 ng/mL of recombinant Human ENO2. No interference was observed.

SPECIES REACTIVITY

This kit recognizes human Neuron-specific Enolase protein.

Other species reactivity was determined by measuring 50 µg/mL brain homogenate tissue extract samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human brain homogenate tissue extract assayed at the same dilution.

100% reactivity was observed in mouse.

Other species reactivity not determined.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g., linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.072	0.071	0.071
312.5	0.150	0.144	0.147
625	0.203	0.191	0.197
1,250	0.346	0.290	0.318
2,500	0.539	0.518	0.529
5,000	0.831	0.921	0.876
10,000	1.500	1.745	1.622
20,000	3.323	3.399	3.361

Table 1. Example of human Neuron-specific Enolase standard curve in Sample Diluent NS. The Neuron-specific Enolase standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.044	0.048	0.046
312.5	0.114	0.112	0.113
625	0.158	0.177	0.167
1,250	0.270	0.292	0.281
2,500	0.489	0.518	0.504
5,000	0.887	0.834	0.860
10,000	1.618	1.522	1.570
20,000	2.966	2.855	2.910

Table 2. Example of human Neuron-specific Enolase standard curve in 1X Cell Extraction Buffer PTR. The Neuron-specific Enolase standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	16	126.7 pg/mL
1X Cell Extraction Buffer PTR	16	136.5 pg/mL

Recovery

Three concentrations of Neuron-specific Enolase were spiked into the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Serum	94	88 - 105
50% Plasma – Citrate	95	83 - 102
50% Plasma – EDTA	103	101 - 104
50% Plasma – Heparin	102	95 - 113
50% Cell Culture Media	103	100 - 105
50% Cerebrospinal Fluid	96	86 - 109
200 µg/mL SH-SY5Y Cell Extract	88	84 - 93
1 µg/mL Brain Cortex Tissue Extract	98	86 - 109
2 µg/mL Hippocampus Tissue Extract	102	96 - 111

*Media is RPMI 1640 containing 10% fetal bovine serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant Neuron-specific Enolase was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	50% Human Plasma (EDTA)	50% Human Plasma (Heparin)	50% Human CSF	50% Cell Culture Media
Undiluted	pg/mL	9,054.4	9,972.0	10,301.7	9,481.5	9,422.3	11,190.2
	% Expected value	100	100	100	100	100	100
2	pg/mL	4,542.6	4,868.4	5,292.4	5,065.8	4,428.3	5,350.4
	% Expected value	100	98	103	107	94	96
4	pg/mL	2,530.2	2,633.1	3,111.8	2,644.1	2,830.4	2,711.9
	% Expected value	112	106	121	112	120	97
8	pg/mL	1,341.6	1,118.7	1,358.5	1,461.4	1,179.8	1,494.0
	% Expected value	119	90	105	123	100	107
16	pg/mL	NL	696.2	NL	735.6	609.0	727.1
	% Expected value	NL	112	NL	124	103	104

NL – Non-Linear

Native Neuron-specific Enolase was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	100 µg/mL SH-SY5Y Cell Extract	12.5 µg/mL Brain Cortex Tissue Extract	125 µg/mL Hippocampus Tissue Extract
Undiluted	pg/mL	13,393.0	20,166.6	11,774.2
	% Expected value	100	100	100
2	pg/mL	6,186.5	10,504.5	6,520.6
	% Expected value	92	104	111
4	pg/mL	3,096.8	5,131.3	3,366.0
	% Expected value	92	102	114
8	pg/mL	1,478.8	2,324.8	1,793.0
	% Expected value	88	92	122
16	pg/mL	735.0	1,117.7	912.2
	% Expected value	88	89	124

Precision

Mean coefficient of variations of interpolated values of Neuron-specific Enolase from a single concentration of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.3	4.4

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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For technical support contact information, visit: www.abcam.com/contactus

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