ab288927 – Human Glutamine Synthetase SimpleStep ELISA® Kit

For the quantitative measurement of Glutamine Synthetase in human tissue extract.
For research use only - not intended for diagnostic use.

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	Storage Condition
Human Glutamine Synthetase Capture Antibody 10X	600 µL	+4°C
Human Glutamine Synthetase Detector Antibody 10X	600 µL	+4°C
Human Glutamine Synthetase 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
Denaturant	500 µL	+4°C
Sample Diluent NS	12 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

Sample Diluent NS is provided but not necessary for this product.

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.
- 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 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 4 mL deionized water and 1 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Cell Extraction Buffer PTR + Enhancer: Prepare 1X Cell Extraction Buffer PTR + Enhancer 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+ Enhancer 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.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

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).

1. Reconstitute the Glutamine Synthetase standard sample by adding the volume of 1X Cell Extraction Buffer PTR + Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 64,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 345 µL of 1X Cell Extraction Buffer PTR + Enhancer into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR + Enhancer 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**	39	345	64,000	6,500
2	Standard#1	150	150	6,500	3,250
3	Standard#2	150	150	3,250	1,625
4	Standard#3	150	150	1,625	812.5
5	Standard#4	150	150	812.5	406.3
6	Standard#5	150	150	406.3	203.1
7	Standard#6	150	150	203.1	101.6
8	Blank Control	0	150	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Human Brain Cortex Cell Extract	0.0391 – 1.25 µg/mL
Human Brain Hippocampus Cell Extract	0.0391 – 1.25 µg/mL

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 + Enhancer. 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. At this point, extract samples can be aliquoted and stored at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. To 17 volume parts of extract, add 1 volume part of Denaturant. Mix thoroughly and gently. Incubate samples at room temperature for 10 minutes. Dilute samples 20-fold in 1X Cell Extraction Buffer PTR. Mix thoroughly and gently. Dilute samples further to desired concentration in 1X Cell Extraction Buffer PTR + Enhancer.

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 Step 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:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

For technical support contact information, visit:
https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)

Version 2b | 12-May-26

ab288927 – Human Glutamine Synthetase SimpleStep ELISA® Kit

Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Glutamine Synthetase.

Native signal was detected in tissue extract.

Serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, urine, milk, CSF and cell extract samples have not been tested with this kit.

SPECIES REACTIVITY

This kit is incompatible with mouse and rat tissue extract samples.

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 an 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	0.045	0.045	0.045
101.6	0.099	0.112	0.105
203.1	0.185	0.177	0.181
406.3	0.305	0.256	0.281
812.5	0.581	0.472	0.527
1,625.0	1.104	0.889	0.996
3,250.0	2.131	1.630	1.880
6,500.0	3.538	2.929	3.233

Table 1. Example of human Glutamine Synthetase standard curve in 1X Cell Extraction Buffer PTR + Enhancer. The Glutamine Synthetase standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 18 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=8) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Two concentrations of Glutamine Synthetase were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
0.625 µg/mL Human Brain Cortex Extract	113	109-117
0.625 µg/mL Human Brain Hippocampus Extract	120	119-121

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.

Native Glutamine Synthetase was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR + Enhancer.

Dilution Factor	Interpolated value	1.25 µg/mL Human Cortex Extract	1.25 µg/mL Human Hippocampus Extract
Undiluted	pg/mL	4634	4751
	% Expected value	100%	100%
2	pg/mL	2224	2242
	% Expected value	96%	94%
4	pg/mL	1133.9	1124.4
	% Expected value	98%	95%
8	pg/mL	528.5	555.8
	% Expected value	91%	94%
16	pg/mL	274.1	280.9
	% Expected value	95%	95%

Precision

Mean coefficient of variations of interpolated values of Glutamine Synthetase from three concentrations of human brain cortex extract within the working range of the assay.

	Intra-assay	Inter-assay
N=	**8**	**3**
CV (%)	**4.2**	**6.8**

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

For technical support contact information, visit:
https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)

Version 2b | 12-May-26