

ab283966 – Human Glutathione Synthetase ELISA Kit

For the quantitative measurement of human plasma, serum, urine, saliva, milk, and CSF samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab283966

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the Standard, SP Conjugate and Biotinylated Antibody which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

| Item | Quantity | Storage Condition |
|--|-----------|-------------------|
| 100X Streptavidin-Peroxidase Conjugate | 80 µL | -20°C |
| 10X Diluent N Concentrate | 30 mL | 4°C |
| 1X Standard Diluent | 2 mL | 4°C |
| 20X Wash Buffer Concentrate | 2 x 30 mL | 4°C |
| 50X Biotinylated Human Glutathione Synthetase Antibody | 1 vial | -20°C |
| Chromogen Substrate | 7 mL | 4°C |
| Glutathione Synthetase Standard | 1 vial | -20°C |
| Human Glutathione Synthetase Microplate | 96 wells | 4°C |
| Sealing Tapes | 3 units | 4°C |
| Stop Solution | 11 mL | 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 nm

Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)

Deionized or distilled reagent grade water

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

Prepare only as much reagent as is needed on the day of the experiment.

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent N Concentrate: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Diluent N Concentrate 1:10 with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.

50X Biotinylated Human Glutathione Synthetase Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with Diluent N to produce a 1x solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution.

100X Streptavidin-Peroxidase Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent N to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.

Prepare serially diluted standards immediately prior to use.

Any remaining standard should be stored at -20°C after reconstitution and used within 30 days. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

Reconstitution of the Glutathione Synthetase Standard vial to prepare a 16 ng/mL Stock Standard.

- First consult the Glutathione Synthetase Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X Diluent N to add when resuspending the Glutathione Synthetase Standard vial to produce an 16 ng/mL Stock Standard by using the following equation:
 - o C_s = Starting mass of Glutathione Synthetase Standard (see vial label) (ng)
 - o C_f = The 16 ng/ml Glutathione Synthetase Stock Standard final required concentration
 - o V_d = Required volume of 1X Diluent N for reconstitution (µL)
 - o Calculate total required volume 1X Diluent N for resuspension:

$$(C_s / C_f) \times 1,000 = V_d$$

Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_s = 8 ng of Glutathione Synthetase Standard in vial

C_f = 16 ng/mL Glutathione Synthetase Standard Stock concentration

V_d = Required volume of 1X Diluent N for reconstitution (8 ng / 16 ng/mL) x 1,000 = 500 µL

- Reconstitute the Glutathione Synthetase Standard vial by adding the appropriate calculated amount V_d of 1X Diluent N to the vial to generate the 16 ng/mL Glutathione Synthetase Stock Standard. Mix gently and thoroughly.
- Allow the reconstituted 16 ng/mL Glutathione Synthetase Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label tubes #1 – 8.
- Add 120 µL of 1X Diluent N to tube #1 – 8.
- To prepare Standard #1, add 120 µL of the Stock Standard into tube #1 and mix gently.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent N serves as the zero standard (0 ng/mL).

| Standard # | Volume to dilute (µL) | Volume 1X Diluent N (µL) | Glutathione Synthetase (ng/ml) |
|------------|-------------------------------|--------------------------|--------------------------------|
| 1 | 120 (Stock Standard 16 ng/ml) | 120 | 8.0 |
| 2 | 120 (Standard #1) | 120 | 4.0 |
| 3 | 120 (Standard #2) | 120 | 2.0 |
| 4 | 120 (Standard #3) | 120 | 1.0 |
| 5 | 120 (Standard #4) | 120 | 0.5 |
| 6 | 120 (Standard #5) | 120 | 0.25 |
| 7 | 120 (Standard #6) | 120 | 0.125 |
| 8 | - | 120 | 0.0 |

Sample Preparation

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 1:16 sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 1:16 sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles

Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x or 2x - 10x into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 1:4 sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 1:20 sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

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, standard solutions, and samples as instructed. Bring all reagents to RT before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimise exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µl of Glutathione Synthetase Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µl of Biotinylated Human Glutathione Synthetase Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µl of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µl of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue colour density develops.
10. Add 50 µl of Stop Solution to each well. The colour will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

TYPICAL DATA

Typical data provided for demonstration purposes only.

| Standard # | ng/mL | Average OD |
|------------|-------|------------|
| 1 | 8.0 | 2.381 |
| 2 | 4.0 | 1.908 |
| 3 | 2.0 | 1.268 |
| 4 | 1.0 | 0.778 |
| 5 | 0.5 | 0.474 |
| 6 | 0.25 | 0.316 |
| 7 | 0.125 | 0.231 |
| 8 | 0.0 | 0.138 |

PERFORMANCE CHARACTERISTICS

1. This assay recognizes both natural and recombinant human GSS.
2. The minimum detectable dose of human GSS as calculated by 2SD from the mean of a zero standard was established to be 74 pg/ml.
3. Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
4. Inter-assay precision was determined by testing three plasma samples in twenty assays.

| | Intra-Assay Precision | Inter-Assay Precision |
|--------|-----------------------|-----------------------|
| CV (%) | 5.0 | 9.5 |

RECOVERY

| | |
|----------------------|-----------------|
| Standard Added Value | 0.5 – 4.0 ng/ml |
| Recovery % | 93 – 108 |
| Average Recovery % | 99 |

LINEARITY

| Average Percentage of Expected Value (%) | | |
|--|--------|-------|
| Sample Dilution | Plasma | Serum |
| 1:8 | 102 | 105 |
| 1:16 | 99 | 101 |
| 1:32 | 96 | 97 |

CROSS REACTIVITY

| Species | Cross-Reactivity (%) |
|---------|----------------------|
| Canine | None |
| Bovine | None |
| Equine | None |
| Monkey | 100 |
| Mouse | None |
| Rat | None |
| Swine | 40 |
| Rabbit | None |

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For all technical or commercial enquiries please go to:

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Version 3b | 26 August 2025