NOTE: Revisions to
"Preparation of Reagents"

Product Manual

Glycine Assay Kit

Catalog Number

MET-5070 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Glycine is an amino acid containing amine (-NH2) and carboxyl (-COOH) functional groups, as well as a single hydrogen as its side-chain. Glycine is a non essential amino acid since it is synthesized in the body from the amino acid serine. In most organisms, the enzyme serine hydroxymethyltransferase uses the cofactor pyridoxal phosphate to convert serine (in the presence of tetrahydrofolate) to glycine and methylene tetrahydrofolate. In the vertebrate liver, glycine synthase is used to convert methylene tetrahydrofolate and NADH to glycine, tetrahydrofolate, and NAD+.

Glycine is one of 20 natural amino acids that serve as fundamental building blocks for the production of proteins. In conjunction with hydroxyproline, glycine makes up the helical structure of collagen. In higher eukaryotes, glycine and succinyl-CoA are used by aminolevulinic acid synthase to make δ-aminolevulinic acid, the main precursor to porphyrins. In the central nervous system, glycine serves as an inhibitory neurotransmitter in the spinal cord, acting as an NMDA receptor co-agonist with glutamate. Glycine supplements have been shown to improve sleep quality, and glycine used in conjunction with other common drugs has been proven beneficial in treating schizophrenia. Bile acids, which are steroid acids found predominantly in the bile of mammals and other vertebrates, are conjugated with glycine (as well as taurine) in the liver, forming primary bile acids.

Cell Biolabs' Glycine Assay Kit is a simple fluorometric assay that measures the amount of free glycine present in foods or biological samples in a 96-well microtiter plate format. Glycine found in polypeptide chains (peptides and proteins) or other conjugated molecules (primary bile acids) are not detected. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, glycine standards and unknown samples. Sample glycine concentrations are determined by comparison with a known glycine standard. The kit has a detection sensitivity limit of 1.56 µM glycine.

Assay Principle

Cell Biolabs' Glycine Assay Kit measures glycine within food or biological samples. Glycine is deaminated by glycine oxidase into glyoxylate plus ammonia and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are read with a standard 96-well fluorometric plate reader. Samples are compared to a known concentration of glycine standard within the 96-well microtiter plate format (Figure 1).

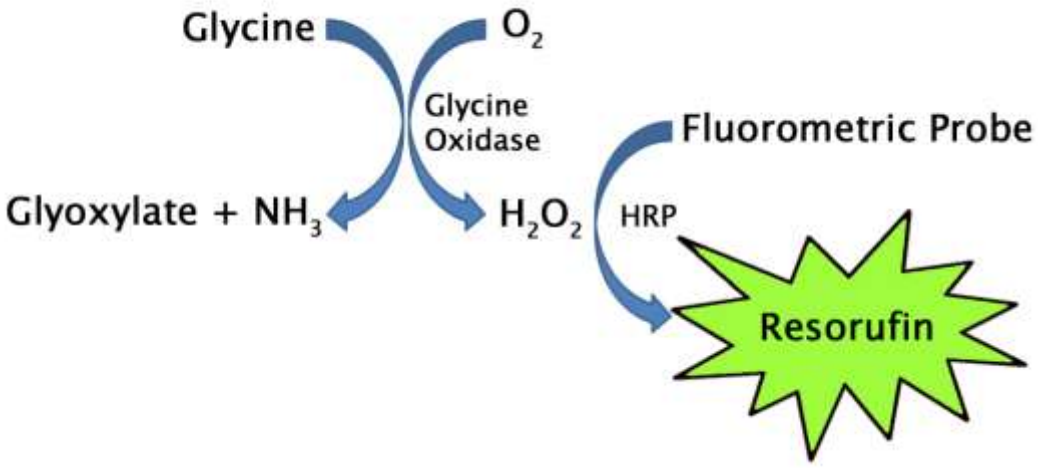

Figure 1. Glycine Assay Principle.

Related Products

1. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
2. MET-5007: Cholic Acid ELISA Kit
3. MET-5008: Chenodeoxycholic Acid ELISA Kit
4. MET-5053: Total Thiol Assay Kit (Colorimetric)
5. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
6. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
7. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
8. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
9. STA-631: Total Bile Acid Assay Kit (Colorimetric)
10. STA-670: Homocysteine ELISA Kit
11. STA-674: Glutamate Assay Kit
12. STA-675: Hydroxyproline Assay Kit
13. MET-5071: Taurine Assay Kit

Kit Components

1. Glycine Standard (Part No. 50701C): One 50 µL tube at 10 mM.
2. 20X Assay Buffer (Part No. 50702A): One 1.5 mL tube.
3. Fluorometric Probe (Part No. 50231C): One 50 µL amber tube.
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol.
5. Glycine Oxidase (Part No. 50703D): Two 1.25 mL tubes containing recombinant Glycine Oxidase H244K from *Bacillus subtilis*.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Microcentrifuge tubes
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Standard 96-well black microtiter plate and/or cell culture microplate
7. Multichannel micropipette reservoir
8. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.

Storage

Upon receipt, store the Glycine Oxidase at -80ºC. Store all other components at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 20X Assay Buffer to 1X with deionized water. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and Glycine Oxidase 1:2 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 0.5 mL of Glycine Oxidase to 488 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Saliva or Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant 1:10 to 1:20 in 1X Assay Buffer just prior to performing the assay.
- Serum or Plasma: Deproteinate the sample by running it through a centrifugal filter unit (e.g. Amicon Ultra 0.5 mL 10K Cat. No. UFC501024) and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant 1:10 to 1:20 in 1X Assay Buffer just prior to performing the assay.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Glycine Standards before use by diluting in 1X Assay Buffer according to Table 2 below.

Standard Tubes	10 mM Glycine Solution (µL)	1X Assay Buffer (µL)	Glycine (µM)
1	5	495	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.56
8	0	250	0

Table 2. Preparation of Glycine Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each Glycine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30 to 60 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of glycine within samples by comparing the sample fluorescence to the standard curve.

Example of Results

The following figures demonstrate typical Glycine Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

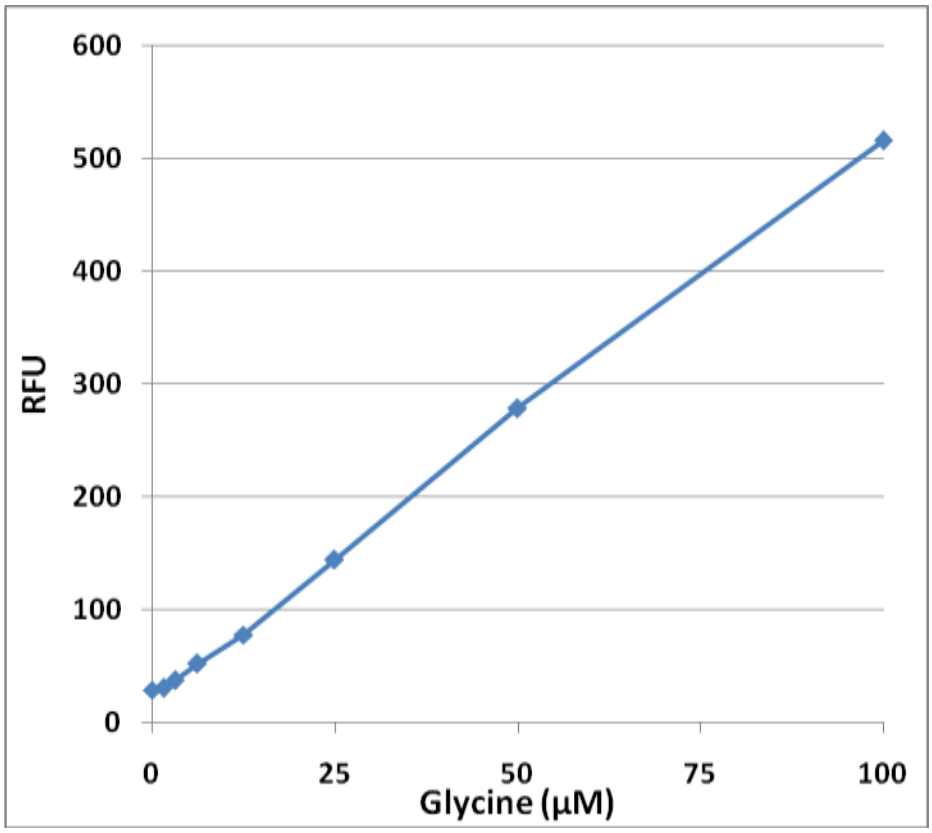

Figure 2: Glycine Standard Curve.

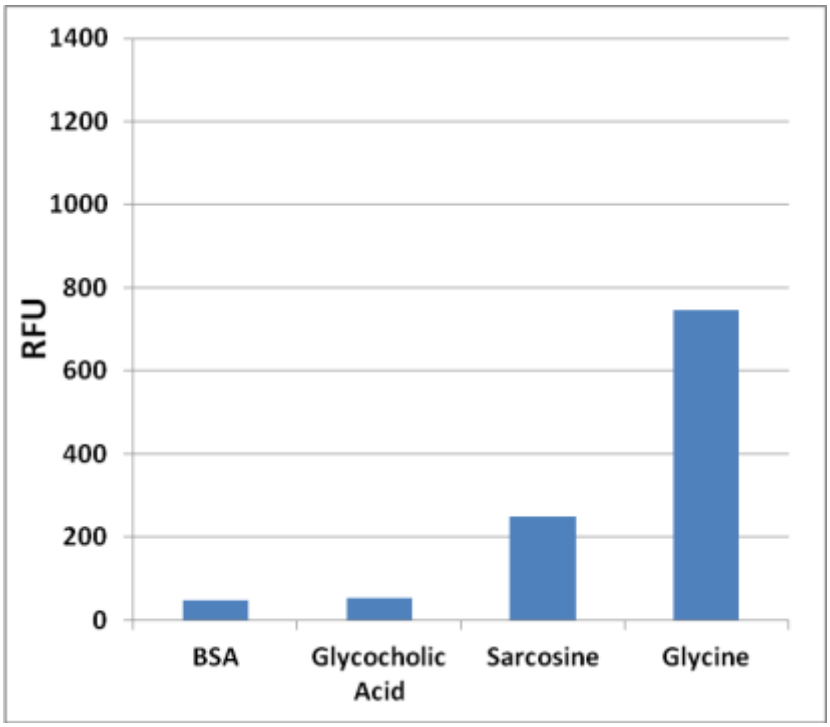

Figure 3: Specificity of Glycine Detection. 10 mg/mL BSA, 50 µM Glycocholic Acid, 50 µM Sarcosine, or 50 µM Glycine were tested according to the Assay Protocol.

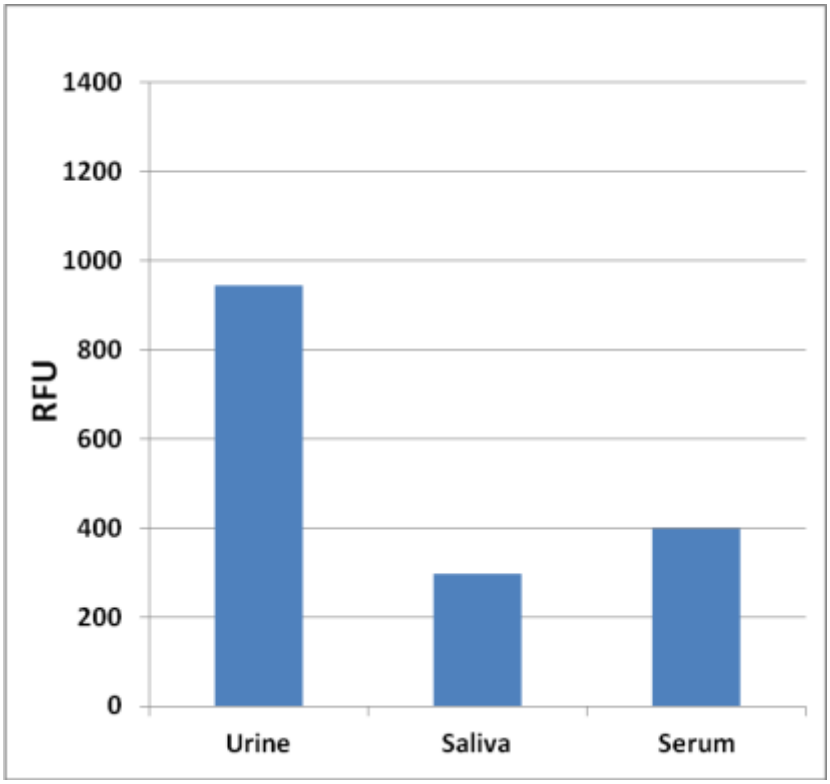

Figure 4: Glycine Detection in Human Urine, Saliva, or Serum using the Glycine Assay Kit. Human samples were diluted 1:20 into 1X assay buffer (serum was deproteinated prior to dilution) according to the Preparation of Samples section.

References

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5. Coyle JT; G Tsai (2004) *Psychopharmacol.* **174**: 32–28.
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Recent Product Citations

1. Lukić, J. et al. (2021). Enrichment of Larval Fish Feed with Free Amino Acids and Proteins by Coating with Lactobacillus paracasei subsp. paracasei BGHN14 Homogenate. *Turkish J. Fish. Aquat. Sci.* **21**(11):569-573. doi: 10.4194/1303-2712-v21_11_05.
2. Escande-Beillard, N. et al. (2020). Loss of PYCR2 Causes Neurodegeneration by Increasing Cerebral Glycine Levels via SHMT2. *Neuron*. doi: 10.1016/j.neuron.2020.03.028.
3. Yang, Z. et al. (2019). Serum Metabolomic Profiling Identifies Key Metabolic Signatures Associated With Pathogenesis of Alcoholic Liver Disease in Humans. *Hepatol Commun*. **3**(4):542-557. doi: 10.1002/hep4.1322.

Warranty

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