



Glycine Assay kit

ARG83368 Glycine Assay kit is an assay kit for Glycine in Serum, plasma, saliva, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83368

Package: 100 assay

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Glycine is the simplest (and the only achiral) proteinogenic amino acid, with a hydrogen atom as its side chain. It has a role as a nutraceutical, a hepatoprotective agent, an EC 2.1.2.1 (glycine hydroxymethyltransferase) inhibitor, a NMDA receptor agonist, a micronutrient, a fundamental metabolite and a neurotransmitter. It is an alpha-amino acid, a serine family amino acid and a proteinogenic amino acid. It is a conjugate base of a glycinium. It is a conjugate acid of a glycinate. It is a tautomer of a glycine zwitterion.

PRINCIPLE OF THE ASSAY

ARG83368 Glycine Assay Kit measures Glycine content within food and 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 probe. Horseradish peroxidase (HPR) catalyzes the reaction between the probe and hydrogen peroxide. The intensity of the color is measured at a wavelength of 540 nm. The concentration of Glycine in the sample is then determined by comparing the O.D. of samples to the standard curve.

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MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Glycine Oxidase at $\leq -80^{\circ}\text{C}$.

Store other component at -20°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (10.0 mM)	50 μL	4°C
20X Assay Buffer	1.5 ml	4°C
Probe	50 μL	-20°C
HRP-Streptavidin Solution	100 μL	-20°C
Glycine Oxidase	1.25 mL X 2	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Store Glycine Oxidase at $\leq -80^{\circ}\text{C}$. Store other component at -20°C at all times. Use the kit before expiration date. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at $1000 \times g$. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at $1000 \times g$. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

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Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

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REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at 4°C.
- **Reaction Mix:** Dilute the Probe at 1:100, HRP at 1:500 and Glycine Oxidase at 1:2 in 1X Assay Buffer. For 20 assays, mix 10 μ L Probe, 2 μ L HRP and 0.5 mL Glycine Oxidase to 488 μ L 1X Assay Buffer for total volume of 1 mL. Store the Reaction Mix at 4°C for 1 day.
- **Standards:** Add 5 μ L of 10.0 mM stock standard into 495 μ L 1X Assay Buffer to generate a standard with 100 μ M of Glycine. Dilute the standards with 1X Assay Buffer serves as zero standard (blank standard, 0 μ M). The example of the standards dilution table is as below:

Standard	Glycine (μ M)	Volume of Assay Buffer (μ L)	Volume of Glycine (μ L)
S1	100	495	5 (10.0 mM stock)
S2	50	250	250(S1)
S3	25	250	250(S2)
S4	12.5	250	250(S3)
S5	6.25	250	250(S4)
S6	3.13	250	250(S5)
S7	1.56	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

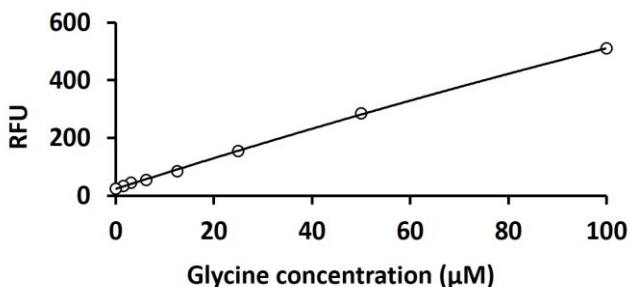
1. Add **50 μ l** of **standard** and **sample** to each wells.
2. Add **50 μ L** of **Reaction Mix** to **standard** each wells.
3. Mix well and Incubate for 30 min- 60 min at 37°C.

Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.

4. Read O.D. with a microplate reader at **540 nm** immediately.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Glycine ranged from 1.56-100 μ M.

The mean MDD was 1.0 μ M