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Product Information

Glycine Assay Kit

Catalog Number **MAK261**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Glycine (Gly) is a non-essential amino acid used to help create muscle tissue and convert glucose into energy. The basic functions of glycine include the participation in the synthesis of creatine, glutathione, heme groups, and conjugated bile acids (bile salts). It is also used in the synthesis of DNA and RNA. It is one of the most abundant residues in the triple-helical structure of collagen, which contributes to the elasticity of skin and connective tissue. It acts as a glucogenic amino acid regulating blood sugar levels. Glycine is necessary for central nervous system function. It possesses both inhibitory and excitatory neurotransmitter functions in the brain stem and spinal cord. Recent studies suggest glycine may play a role in tumorigenesis and malignancy.

The Glycine Assay Kit is a simple and reliable high throughput assay that detects the physiological concentration of glycine in a variety of biological fluids. In the assay, glycine is oxidized generating a fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product proportional to the glycine present. The assay is specific, and other standard and non-standard amino acids do not interfere with the assay. The kit is sensitive to 1 μM of glycine.

This kit is suitable for use with plasma, serum, urine, and other biological samples.

Components
The kit is sufficient for 100 assays in 96 well plates.

Glycine Assay Buffer 25 mL
Catalog Number MAK261A

Glycine Probe 0.4 mL
Catalog Number MAK261B

Glycine Enzyme Mix 1 vl
Catalog Number MAK261C

Glycine Developer 1 vl
Catalog Number MAK261D

Glycine Standard 1 vl
Catalog Number MAK261E

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays
- Spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter
- 10% Glycerol - Molecular Biology Grade (G5516)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glycine Assay Buffer – Allow buffer to come to room temperature before use. Store at –20 °C.

Glycine Probe – Allow probe to come to room temperature before use. Store protected from light at –20 °C. Upon thawing, the Glycine Probe is ready-to-use as supplied.

Glycine Enzyme Mix - Dilute 10 μL of 100% Glycerol with 90 μL of Glycerol Assay Buffer. Vortex for 30 seconds. Reconstitute the Glycine Enzyme Mix with 55 μL of Glycine Assay Buffer with 10% Glycerol. Mix well, then aliquot and store at –20 °C, protected from light. Keep on ice during use and limit to two freeze/thaw cycles.

Glycine Developer - Reconstitute with 220 µL of Glycine Assay Buffer. Aliquot and store at –20 °C. Keep on ice during use and limit to one freeze/thaw cycle.

Glycine Standard - Reconstitute with 100 µL of water to generate a 100 mM Glycine Standard. Dissolve completely. Store at –20 °C and use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glycine Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM Glycine Standard with 990 µL of water to prepare a 1 mM standard solution. Further dilute to 50 µM by adding 50 µL of 1 mM Glycine Standard to 950 µL of water. Add 0, 2, 4, 6, 8, and 10 µL of the 50 µM Glycine standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add Glycine Assay Buffer to each well to bring the final volume to 50 µL.

Sample Preparation
The fluorometric assay requires 50 µL of sample for each reaction (well).

Homogenize tissue (10–20 mg) or lyse cells (1×10^6) in 100 µL of Glycine Assay Buffer. Centrifuge the samples at 10,000 × *g* at 2–8 °C for 5 minutes to remove insoluble material. Collect the supernatant.

Centrifuge liquid samples at 10,000 × *g* at 2–8 °C for 5 minutes.

Add 1–50 µL samples into wells of a 96 well plate. Bring samples to a final volume of 50 µL with Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve. Glycine concentration varies over a wide range depending on the sample. For example - human urine: 44-300 µM/mM creatinine; human serum: 126–490 µM glycine; saliva: 10–300 µM glycine. The recommended dilution factor for urine is 50–500×, for serum is 16-250×, and for saliva is 4-50×.

Metabolites found in biological samples can interfere with the assay. Dilute biological samples with Glycine Assay Buffer. If interference is observed in the diluted samples, prepare parallel sample well(s) as sample background control(s) by omitting the Glycine Enzyme Mix.

For samples with high protein content, deproteinize using a 10 kDa MWCO spin filter.

To ensure accurate determination of Glycine in the test samples or for samples having low concentrations of glycine, we recommend spiking samples with a known amount of Glycine Standard (e.g., 0.3 nmole).

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

2. Dilute the Glycine Enzyme Mix 10-fold. (Dilute 2 µL of Glycine Enzyme Mix with 18 µL of Glycine Assay Buffer). 5 µL will be needed for each reaction (well) to be assayed.

Table 1.
Master Reaction Mix

Reagent	Samples and Positive Control	Background Control Mix
Glycine Assay Buffer	42 µL	47 µL
Glycine Enzyme Mix (diluted)	5 µL	–
Glycine Developer	2 µL	2 µL
Glycine Probe	1 µL	1µL

3. Add 50 µL of the Master Reaction Mix to each sample and positive control well containing the Glycine standard. If using a sample control, add 50 µL of Sample Control Mix to sample control reactions (wells). Mix well using a horizontal shaker or by pipetting.

4. Incubate the plate for 1 hour at 25 °C. Protect the plate from light during the incubation.

5. Measure fluorescence intensity (λ_{ex} = 535 nm/ λ_{em} = 587 nm).

Results

Calculations

The background is the value obtained for the 0 (assay blank) Glycine Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Subtract the Sample Background Control value from the sample readings.

Use the values obtained from the appropriate Glycine standards to plot a standard curve. The amount of glycine present in the samples may be determined from the standard curve.

Notes: A new standard curve must be set up each time the assay is run.

For spiked samples, correct for interference by using the following equation:

$$\frac{RFU_{sample\ (corrected)}}{(RFU_{sample+Gly\ Std\ (corrected)}) - (RFU_{sample\ (corrected)})} * Gly\ spike\ (nmol)$$

Concentration of Glycine

$$S_a/S_v = C$$

S_a = Amount of Glycine in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Glycine in sample

Glycine molecular weight: 75.07 g/mole

Sample Calculation

Amount of Glycine (S_a) = 0.384 nmole (from standard curve)
Sample volume (S_v) = 50.0 μL

Concentration of Glycine in sample

0.384 nmole/50.0 μL = 0.00768 nmole/μL

0.00768 nmole/μL x 75.07 ng/nmole = 0.577 ng/μL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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