Version 4a, Last updated 6 June 2025

ab211100 Glycine Assay Kit (Fluorometric)

For the sensitive and accurate measurement of Glycine in various biological samples.

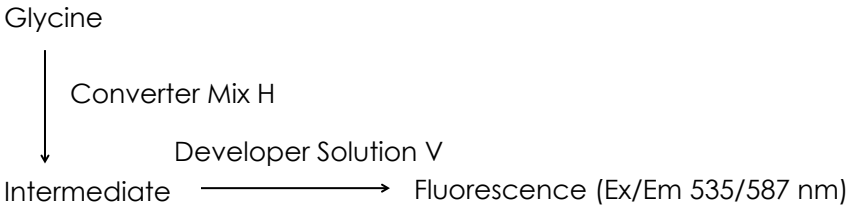
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Glycine Assay Kit (Fluorometric) (ab211100) provides a provides a simple, sensitive, and high-throughput adaptable assay that detects physiological concentrations of glycine (GLY) in multiple biological samples such as cell and tissue lysates and biological fluids. In this assay, Glycine is oxidized in the presence of Converter Enzyme Mix XI is converted to an intermediate, which reacts with the fluorescent probe to generate a strong stable signal at Ex/Em = 535/587 nm. The intensity of the signal is directly proportional to the amount of GLY in the sample.

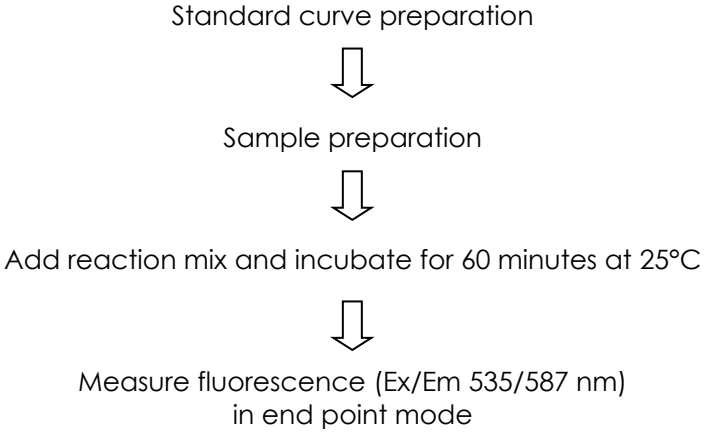
The reaction is specific and other amino acids do not interfere with the assay. The assay can detect as little as 1 µM of Glycine in a variety of biological samples.



Glycine (GLY, G) is one of the 20 standard amino acids commonly found in proteins. Glycine's side chain is a hydrogen substituent, which makes it the smallest proteogenic and the only non-chiral amino acid. Basic functions of Glycine include the participation in the synthesis of creatine, glutathione, heme groups, and conjugated bile acids (bile salts). It is also present as one of the most abundant residues in the triple-helical structure of collagen.

Glycine acts as a glucogenic amino acid by regulating sugar levels in blood. Therefore, glycine supplementation has been used in patients suffering anemia, hypoglycemia and chronic fatigue. Glycine possesses both inhibitory and excitatory neurotransmitter functions in the brain stem and spinal cord. In cancer cells, glycine consumption is highly correlated to cancer cell proliferation via purine synthesis. Glycine uptake in cancer cell studies supports the role of this amino acid in tumorigenesis and malignancy.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 58	25 mL	-20°C	-20°C
OxiRed™ Probe	2 x 0.2 mL	-20°C	-20°C
Converter Enzyme XI	1 vial	-20°C	-20°C
Glycine Standard	1 vial	-20°C	-20°C
Developer Solution V	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 58 was previously labelled as Assay Buffer LVIII and GLY Assay Buffer, and OxiRed™ Probe as OxiRed Probe and GLY Probe. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- 100% Gycerol (molecular biology grade)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well black plate with flat bottom
- Dounce homogenizer (if using tissue)
- 10kD Spin Column (ab93349): for removal of interfering metabolites present in the sample

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 58:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Prepare 100 µL Assay Buffer 58 containing 10% Glycerol: add 10 µL of 100% glycerol (not supplied) to 90 µL Assay Buffer 58.

9.2 OxiRed™ Probe:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Light sensitive.

9.3 Converter Enzyme Mix XI:

Reconstitute in 55 µL of Assay Buffer 58 containing 10% Glycerol (Step 9.1).

Keep enzyme mix on ice while in use and protected from light (wrap vial in foil). Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Freeze/thaw should be limited to two times.

9.4 Developer Solution V:

Reconstitute in 220 µL of Assay Buffer 58 by pipetting up and down to dissolve completely. Keep on ice while in use. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Freeze/thaw should be limited to one time.

9.5 Glycine Standard:

Reconstitute in 100 µL of ddH_2O to generate a 100 mM standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 1 mM standard by diluting 5 µL of the 100 mM Glycine Standard with 495 µL ddH_2O. Mix well.

10.2 Prepare a 50 µM standard, by diluting 50 µL of the 1 mM Glycine Standard with 950 µL ddH_2O. Mix well.

10.3 Using 50 µM Glycine Standard, prepare standard curve dilutions as described in the table in a microplate or microcentrifuge tubes:

Standard #	Glycine Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount GLY in well (nmol/well)
1	0	150	50	0
2	6	144	50	0.1
3	12	138	50	0.2
4	18	132	50	0.3
5	24	126	50	0.4
6	30	120	50	0.5

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation: 1 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 100 µL of Assay Buffer 58 on ice.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Incubate cell homogenate for 10 minutes on ice.
11.1.6 Centrifuge 5 minutes at 10,000 x*g* in a cold microcentrifuge at 4°C to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new tube.
11.1.8 Keep on ice.
11.1.9 Cell lysates may contain enzymes that can interfere with the assay. Remove these enzymes from the samples by using a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 minutes at 4°C. Collect the filtrate.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10-20 mg).
11.2.2 Wash tissue in cold PBS.
11.2.3 Homogenize tissue in 100 µL of Assay Buffer 58 with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
11.2.4 Incubate homogenate for 10 minutes on ice.
11.2.5 Centrifuge 5 minutes at 4°C at 10,000 x*g* in a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a new tube.
11.2.7 Keep on ice.
11.2.8 Tissue samples may contain enzymes that can interfere with the assay. Remove these enzymes from the sample by using a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 minutes at 4°C. Collect the filtrate.

11.3 Biological fluids (plasma, serum, urine):

11.3.1 Centrifuge 5 minutes at 4°C at 10,000 x*g* in a cold microcentrifuge to remove any insoluble material.
11.3.2 Collect supernatant and transfer to a new tube.
11.3.3 Keep on ice.
11.3.4 For samples have high protein content (such as urine), we recommend deproteinizing the samples with a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 minutes at 4°C. Collect the filtrate.

Average glycine concentrations in human biological fluids are:

Sample	Glycine Concentration	Recommended Dilution factor
Human urine	44-300 µM/mM creatinine	1:50-500
Human serum	126-490 µM	1:16-250
Human saliva	4-50 µM	1:4-50

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.
Δ Note: To ensure accurate determination of Glycine in samples having low concentrations of glycine, we recommend spiking samples with a known amount of Glycine Standard (0.3 nmol).

12. 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.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: Metabolites found in biological samples can interfere with the assay. Set up Sample Background Controls to correct for background noise.

12.1 Set up reaction wells:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 58).
- Background Control Sample wells =1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 58).

12.2 GLY Reaction preparation:

12.2.1 Prepare a 1:10 dilution of Converter Enzyme Mix XI/(ie., 2 µL Converter Enzyme Mix XI + 18 µL Assay Buffer 58) immediately prior to use.

12.2.2 Prepare 50 µL of Reaction and background Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 58	42	47
Diluted Converter Enzyme Mix XI	5	-
Developer Solution V	2	2
OxiRed™ Probe	1	1

12.2.3 Add 50 µL of Reaction Mix into each standard and sample wells. Mix well.

12.2.4 Add 50 µL of Background Reaction Mix into the background control sample wells. Mix well.

12.3 Plate measurement:

12.3.1 Incubate plate at 25°C for 60 minutes protected from light.

12.3.2 Measure output on a fluorescent microplate reader at Ex/Em = 535/587 nm in end point mode.

13. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

13.1 If significant, subtract the sample background control from sample reading.

13.2 Average the duplicate reading for each standard and sample.

13.3 Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence (RFU).

13.4 Plot the corrected fluorescence values for each standard as a function of the final concentration of Glycine.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Apply RFU of the sample to the Glycine Standard curve to get Glycine (B) amount in the sample wells.

13.7 Concentration of Glycine in the test samples (nmol/µL or mM) is calculated as:

$$\text{Glycine concentration} = \frac{B}{V} * D$$

Where:

B = amount of Glycine amount in the sample well calculated from standard curve (nmol).

V = sample volume added in the sample wells (µL).

D = sample dilution factor.

Glycine molecular weight: 75 g/mol

13.8 For spiked samples, correct for any sample interference by subtracting the sample reading from the spiked sample reading.

13.9 For spiked samples, the concentration of Glycine in the sample well is calculated as:

$$GLY = \frac{RFUsc}{(RFU_s + Tsc) - (RFUsc)} * GLY\ spike\ (nmol)$$

Where:
RFUsc = RFU sample corrected
RFUs = RFU sample
Tsc = amount of glycine from standard curve corrected

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

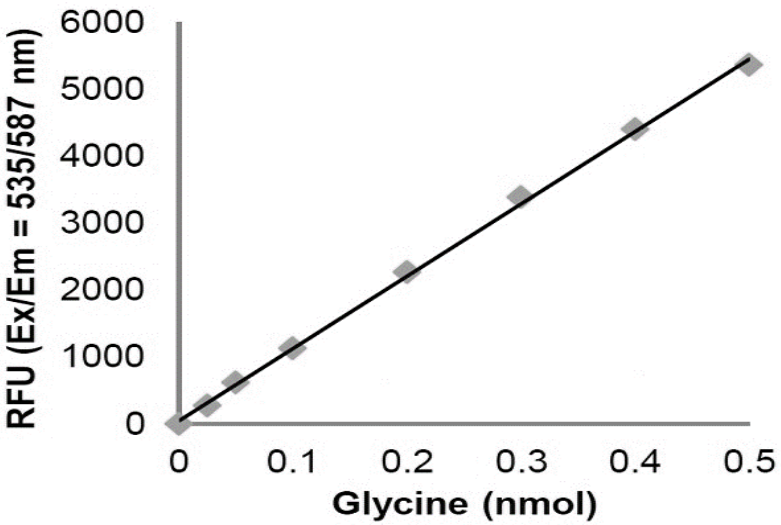


Figure 1. Typical Glycine standard calibration curve.

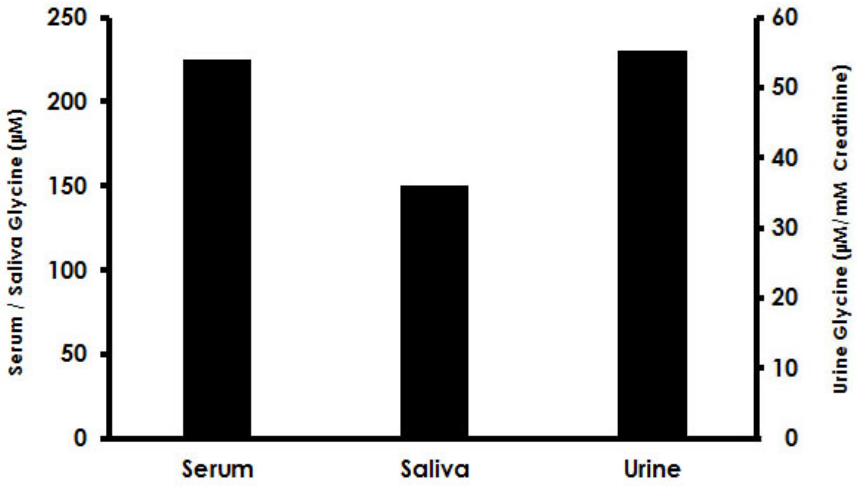


Figure 2. Estimation of Glycine in human serum, saliva, and urine. Samples were deproteinized using 10 kD spin column (ab 93349) and diluted with Assay Buffer 58 prior to performing assay: (serum 1:64 dilution; saliva 1:32 dilution; urine 1:128 dilution). 25 µL of each diluted sample was spiked with 0.3 nmol of Glycine Standard and assayed following the kit protocol. Glycine concentrations in serum (224 ± 21µM), saliva (149 ± 7 µM) and urine (54 ± 4 µM/mM creatinine).

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare Glycine Standard dilution [0.1 – 0.5 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL) and background sample control wells (50 µL).
- Prepare a master mix for Reaction Mix and a master mix for Background Reaction Mix:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 58	42	47
Diluted GLY Enzyme	5	0
Developer Solution V	2	2
OxiRed™ Probe	1	1

- Add 50 µL Reaction Mix to standard and sample wells.
- Add 50 µL Background Reaction Mix to Sample Background control wells.
- Incubate plate at 25°C for 60 minutes protected from light.
- Measure plate at Ex/Em = 535/587 nm in end point mode.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)