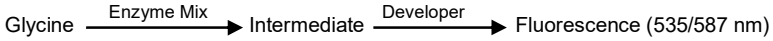
Glycine Assay Kit (Fluorometric) (BN00817)

(Catalog # BN00817; 100 assays; Store at -20°C)

I. Introduction:

Glycine (GLY) is one of the 20 standard amino acids commonly found in proteins. GLY side chain is a hydrogen substituent, thus it is the only amino acid that is not chiral and the smallest among the proteogenic amino acids. 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. It acts as a glucogenic amino acid regulating sugar levels in blood. Therefore, glycine supplementation has been used in patients suffering anemia, hypoglycemia and chronic fatigue. In cancer cells, GLY 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. GLY possesses both inhibitory and excitatory neurotransmitter functions in the brain stem and spinal cord. Assay Genie's Glycine Assay Kit provides a simple, sensitive, and high-throughput adaptable assay that detects physiological concentration of glycine in a variety of biological fluids. The principle of the assay is based on the oxidation of glycine producing a fluorophore (Ex/Em = 535/587 nm) with a stable signal, which is directly proportional to the amount of GLY in the sample. The assay is specific and other standard and non-standard amino acids do not interfere with the assay. The assay can detect as little as 1 µM of Glycine in a variety of samples.



II. Application:

- Estimation of glycine in various biological samples

III. Sample Type:

- Biological fluids such as serum, plasma, saliva, urine, etc.

IV. Kit Contents:

Components	BN00817	Cap Code	Part Number
GLY Assay Buffer	25 ml	WM	BN00817-1
GLY Probe	0.4 ml	Red	BN00817-2
GLY Enzyme Mix	1 vial	Blue	BN00817-3
GLY Developer	1 vial	Green	BN00817-4
GLY Standard	1 vial	Yellow	BN00817-5

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- 10 kDa Spin Column
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **GLY Assay Buffer**: Store at -20°C or 4°C. Bring to room temperature (RT) before use.
- **GLY Probe:** Light sensitive. Store at -20°C. Bring to RT before use.
- **GLY Enzyme Mix:** Reconstitute with 55 µl GLY Assay Buffer containing 10% molecular biology grade glycerol (not provided). Make GLY Assay Buffer containing 10% glycerol by adding 10 µl of 100% Glycerol to 90 µl of GLY Assay Buffer, vortex for 30 sec. Aliquot and store at -20°C. Light sensitive, protect from light. Freeze/thaw should be limited to two times. Keep on ice during use.
- **GLY Developer:** Reconstitute with 220 µl of GLY Assay Buffer. Aliquot and store at -20°C. Freeze/thaw should be limited to one time. Keep on ice during use.
- **Glycine Standard:** Reconstitute with 100 µl of dH_2O to generate 100 mM Glycine Standard. Dissolve completely. Store at -20°C. Use within 2 months.

VII. Glycine Assay Protocol:

1. **Sample Preparation:** Lyse cells (1 x 10^6) or homogenize tissue (~10-20 mg) samples using 100 µl GLY Assay Buffer. Centrifuge cell lysate, tissue homogenate, or biological fluids at 10,000 X g, 4°C for 5 min. Collect the supernatant. Dilute samples using GLY Assay Buffer & add 1-50 µl into desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with GLY Assay Buffer.

   Notes:

   a. Glycine concentration varies over a wide range depending on the sample. Glycine range concentrations in some biological samples are: human urine: 44-300 µM/mM creatinine; human serum: 126-490 µM; saliva: 10-300 µM. Recommended dilution factor: serum (16-250), urine (50-500), saliva (4-50). For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   b. Metabolites found in biological samples interfere with the assay. It is recommended to dilute biological samples with GLY Assay Buffer. If interference is observed in the diluted samples, prepare parallel sample well(s) as sample background control(s) and make up the volume to 50 µl/well with GLY Assay Buffer.

   c. For samples having high protein content, we recommend deproteinizing the samples (tissue or cell lysate or biological fluids) using 10 kDa Spin Column. Add sample to the spin column, centrifuge at 10,000 X g, 4°C for 10 min. Collect the filtrate.

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

2. **Standard Curve Preparation:** Prepare 1 mM Glycine Standard by adding 10 µl of 100 mM GLY Standard to 990 µl of ddH_2O. Further dilute to 50 µM by adding 50 µl of 1 mM Glycine Standard to 950 µl ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 50 µM Glycine Standard into a series of wells in a 96-well plate to generate 0, 0.1, 0.2, 0.3, 0.4 and 0.5 nmol of Glycine/well. Adjust the volume to 50 µl/well with GLY Assay Buffer.
3. **Reaction Mix:** Dilute GLY Enzyme Mix 10-fold (i.e. 2 µl Gly Enzyme Mix + 18 µl GLY Assay Buffer). Mix enough reagents for the total number of wells to be assayed. For each well, prepare 50 µl of Reaction Mix containing:

	Reaction Mix	*Background Control Mix
GLY Assay Buffer	42 µl	47 µl
Diluted GLY Enzyme Mix	5 µl	----
GLY Developer	2 µl	2 µl
GLY Probe	1 µl	1 µl

Mix well. Add 50 µl of Reaction Mix into Standard and sample wells. Mix.

* For samples having background, add Background Control Mix to background control well(s) and mix.

4. **Measurement:** Incubate plate at 25°C for 60 min., protected from light. Measure fluorescence (Ex/Em = 535/587 nm) in end point mode.
5. **Calculation:** Subtract 0 Gly Standard reading from all readings. Plot the Gly Standard Curve. If sample background control is significant, then subtract sample background control reading from sample reading. Apply corrected RFU to Standard Curve to get B nmol Glycine in the sample well.

Sample Glycine Concentration (C) = B/V X D nmol/µl or mM

Where: **B** is amount of Glycine in the sample well from Standard Curve (nmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

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

$$\textbf{Glycine amount in spike sample well (B)} = \frac{\mathrm{RFU}_{\mathrm{sample\ (corrected)}}}{(\mathrm{RFU}_{\mathrm{sample+Gl\ \ Std\ (corrected)}}) - (\mathrm{RFU}_{\mathrm{sample\ (corrected)}})} * \mathrm{Gly\ spike\ (nmol)}$$

Glycine molecular weight: 75 g/mol
1 mM ≡ 1000 µM

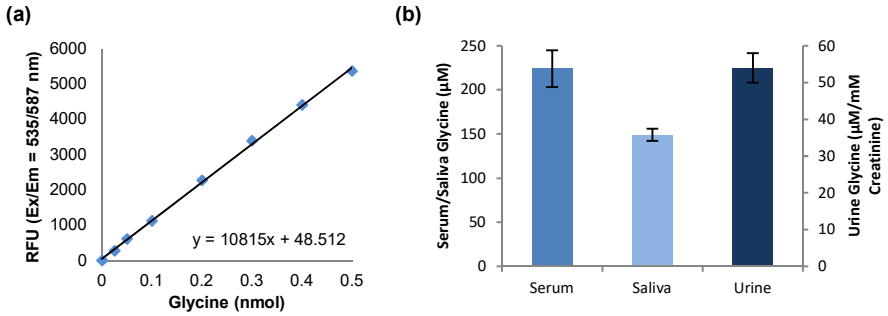


Figure: (a) Glycine Standard Curve. **(b)** Estimation of Glycine concentration in human serum, saliva, and urine. Samples were deproteinized using 10 kDa spin colum and diluted using GLY Assay Buffer (Serum: 64-fold; Saliva: 32-fold; Urine: 128-fold). 25 µl of each diluted sample was spiked with 0.3 nmol of Glycine Standard and assayed following the kit protocol. Glycine concentrations are: Serum: 224 ± 21 µM, Saliva: 149 ± 7 µM, Urine: 54 ±4 µM/mM Creatinine.

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