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Glycine Cleavage System (GCS) Assay Kit (Cat #: E-137)

COMPONENTS: GCS Assay Solution- 5 ml (for 100 wells); store at -80ºC **(shield solution from light during assay)**
Glycine- 0.2 g, store at room temperature
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The GCS assay is based on the reduction of the tetrazolium salt INT in a NADH-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm (molar extinction coefficient = 18 $mM^{-1}cm^{-1}$) and allows for measurement of GCS activity in tissue/cell extracts. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~10^5 washed cells in 50–100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~5 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 3 mg/ml.

Reagent thawing:

Keep thawed GCS Assay Solution on ice and shielded from light. *Do not over thaw.* Freeze solution immediately after use.

Preparation of glycine substrate: Weigh 30 mg glycine and dissolve in 0.2 ml dH_2O by vortexing.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 10 parts of GCS Assay Solution, e.g. 0.1 ml dH_2O mixed with 1 ml GCS Assay Solution.

Reaction solution is prepared by mixing 1 part of freshly prepared glycine substrate and 10 parts of GCS Assay Solution, e.g. 0.1 ml glycine substrate mixed with 1 ml GCS Assay Solution.

Enzyme assay:

1. Add 10 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate.
2. Add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation. Cover plate and incubate in a 37°C incubator for 60 - 90 min. *Do not use CO_2 incubator.* Cherry red color should gradually appear in sample wells.
3. Measure $O.D._{492\ nm}$ using a plate reader.
4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
5. If incubation for 60 min, GCS enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 60 µl/(60 min × 0.4 cm × 18 × 10 µl) = **ΔO.D. × 13.89**. For 90 min, enzyme activity = **ΔO.D. × 9.26.** Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (cat#E-101).

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.