

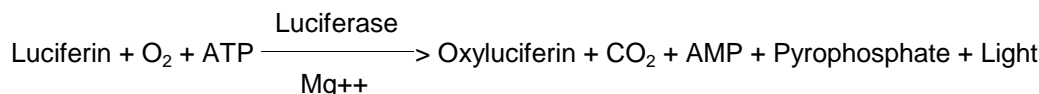


Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of LUCIFERASE (EC 1.13.12.7) from Firefly

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

AMP = Adenosine 5'-Monophosphate

CONDITIONS: T = 25°C, pH = 7.6

METHOD: Luminometric

REAGENTS:

- A. 1 M Glycine-Tris Buffer with 10 mM Ethylenediaminetetraacetic Acid and 100 mM Magnesium Sulfate, pH 7.0 at 25°C (Enz Dil)
(Prepare 50 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS, and Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880. Adjust to pH 7.0 at 25°C with solid Trizma Base, Sigma Prod. No. T-1503.)
- B. 0.15 mM Luciferin, 50 mM glycine, 1 mM Tris, 5 mM MgSO₄, 0.5 mM Ethylenediaminetetraacetic Acid, 0.1% (w/v) Bovine Serum Albumin, and 0.1% (w/v) Sodium Azide
(Prepare by reconstituting 1 vial of Firefly Diluent, Glycine Diluent with added Luciferin, Sigma Prod. No. F-3766 with 10 ml of deionized water. Store on ice.)
- C. 1 mM Adenosine 5'-Triphosphate Solution
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- D. 0.01 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10.0 ml by adding 0.1 ml of Reagent C to 9.9 ml of deionized water.)

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REAGENTS: (continued)

- E. 0.15 mM Luciferin, 50 mM Glycine, 1 mM Tris, 5 mM Magnesium Sulfate, 0.5 mM Ethylenediaminetetraacetic Acid, 0.1% (w/v) Bovine Serum Albumin, 0.1% (w/v) Sodium Azide, and 100 nM Adenosine 5'-Triphosphate Solution (Reaction Cocktail)
(Immediately before use, prepare by adding 0.010 ml of Reagent D to 0.99 ml of Reagent B.)
- F. ^{14}C 1.0 μCi Standard (^{14}C Std)
(Use ^{14}C 1.0 μCi Counting Standard.)¹
- G. Luciferase Enzyme Solution
(Immediately before use, prepare a solution containing 3,000,000 - 4,000,000 units/ml of Luciferase in cold Reagent A.)

PROCEDURE:

Set Reagent F (^{14}C Std) equal to 50% on the 100 scale of a photometer. Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>
Reagent E (Reaction Cocktail)	0.050

Incubate at 25°C for 10 minutes. Then add:

Reagent G (Enzyme Solution)	0.001
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Immediately after adding Reagent G (Enzyme Solution), shake the tube twice and record the peak height of the luminescence.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{Photometer Reading})(df)}{(0.001)}$$

Photometer Reading = One light unit will produce one count at the peak height of luminescence

df = Dilution factor

0.001 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

Light units are measured in 50 μl of assay mixture containing 5 picomoles of ATP and 7.5 nanomoles luciferin in glycine Tris buffer, pH 7.6 at 25°C. One light unit produces a biometer peak height equivalent to 0.02 μCi of ^{14}C in PPO/POPOP cocktail.

FINAL ASSAY CONCENTRATION:

In a 0.051 ml reaction mix, the final concentrations are 0.15 mM luciferin, 49 mM glycine, 0.98 mM Tris, 7 mM magnesium sulfate, 0.7 mM ethylenediaminetetraacetic acid, 0.1% (w/v) bovine serum albumin, 100 nM adenosine 5'-triphosphate and 3,000 - 4,000 units luciferase.

REFERENCE:

Leach, F.R. and Webster, J.J. (1986) *Methods in Enzymology*, 133, Part B, 51-70

Lin, S. and Cohen, H.P. (1968) *Analytical Biochemistry* **24**, 531-540

Strehler, B.L. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Vol. 4, 2112-2121

NOTES:

1. The ^{14}C 1.0 μCi Counting Standard used was obtained from Johnston's Lab Inc. (Prod. No. LS-1). The scintillation cocktail used is PPO(2,5-Diphenyloxazole)/POPOP(1,4-bis[5-Phenyl-2-oxazolyl]-benzene). This standard is no longer available. Other acceptable counting standards may be used.
2. This assay is based on the cited references.

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NOTES: (continued)

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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