

Enzymatic Assay of RIBONUCLEIC ACID POLYMERASE¹
(EC 2.7.7.6)

PRINCIPLE:

DNA + NTP $\xrightarrow{\text{RNA Polymerase}}$ DNA + RNA + PP_i

PP_i + UDPG $\xrightarrow{\text{UDPG Pyrophosphorylase}}$ UTP + Glucose 1-Phosphate

Glucose 1-Phosphate $\xrightarrow{\text{Phosphoglucomutase}}$ Glucose 6-Phosphate

Glucose 6-Phosphate + β-NADP $\xrightarrow{\text{G-6-PDH}}$ 6-PG + β-NADPH

Abbreviations used:

DNA = Deoxyribonucleic Acid

NTP = Nucleotide Triphosphate

RNA = Ribonucleic Acid

PP_i = Inorganic Pyrophosphate

UDPG = Uridine 5'-Diphosphoglucose

UTP = Uridine 5'-Triphosphate

β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

6-PG = 6-Phospho-D-Gluconate

β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

CONDITIONS: T = 37°C, pH = 7.9, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 190 mM Tris Buffer with 75 mM Magnesium Chloride, 450 mM Potassium Chloride and 4 mM Dithiothreitol, pH 7.9 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, Potassium Chloride, Sigma Prod. No. P-3911, and DL-Dithiothreitol, Sigma Prod. No. D-0632. Adjust to pH 7.9 at 37°C with 1 M HCl.)

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REAGENTS: (continued)

- B. 1 M Tris Base Solution
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503.)
- C. 10 mM Nucleotide 5'-Triphosphate Solution (NTP)
(Prepare the Nucleotide 5'-Triphosphate Solution by combining equal volumes of the following components:
1. 40 mM Cytidine 5'-Triphosphate Solution (CTP)
(Prepare 10 ml in deionized water using Cytidine 5'-Triphosphate, Sodium Salt, Sigma Prod. No. C-1381.)
 2. 40 mM Guanosine 5'-Triphosphate Solution (GTP)
(Prepare 10 ml in deionized water using Guanosine 5'-Triphosphate, Lithium Salt, Sigma Prod. No. G-6006.)
 3. 40 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
 4. 40 mM Uridine 5'-Triphosphate Solution (UTP)
(Prepare 10 ml in deionized water using Uridine 5'-Triphosphate, Sodium Salt, Sigma Prod. No. U-6625.)

Adjust to pH 7.9 with a small volume of Reagent B.
PREPARE FRESH.)

- D. 10 mM Tris Buffer with 0.1 mM Ethylenediaminetetraacetic Acid, pH 7.9 at 25°C (DNA Buffer)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS. Adjust to pH 7.9 at 25°C with 1 M HCl.)
- E. 0.05% (w/v) Deoxyribonucleic Acid with 0.5% (w/v) Bovine Serum Albumin Solution (DNA/BSA)²
(Prepare 50 ml in Reagent D using Deoxyribonucleic Acid (DNA), Sigma Prod. No. D-1501, and Albumin, Bovine, Essentially Fatty Acid Free, Sigma Prod. No. A-6003.)

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REAGENTS: (continued)

- F. 90 mM Uridine 5'-Diphosphoglucose with 2 mM Glucose 1,6-Diphosphate Solution³ (UDPG/G1,6DP)
(Prepare 3 ml in deionized water using Uridine 5'-Diphosphoglucose, Disodium Salt, Sigma Prod. No. U-4625, and α -D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Hydrate, Sigma Prod. No. G-7137.)
- G. 10 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310 in the appropriate volume of deionized water **or** prepare 2 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH.**)
- H. 20 mM Sodium Phosphate Solution, pH 7.5 at 25°C.
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.5 at 25°C with 1 M NaOH.)
- I. 50% (v/v) Glycerol Solution
(Prepare 200 ml in Reagent H using Glycerol, Sigma Prod. No. G-9012.)
- J. Phosphoglucomutase/Glucose-6-Phosphate Dehydrogenase⁴ Solution (PGM/G-6-PDH)
(Prepare a solution containing 500 units/ml of Phosphoglucomutase, Sigma Prod. No. P-3397, and 500 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-7877 in Reagent I.)⁵
- K. Uridine-5'-Diphosphoglucose Pyrophosphorylase Solution (UDPG-PP)
(Prepare a solution containing 100 units/ml of Uridine-5'-Diphosphoglucose Pyrophosphorylase, Sigma Prod. No. U-8501, in cold Reagent I.)
- L. 100 mM Manganese Chloride Solution ($MnCl_2$)
(Prepare 10 ml in deionized water using Manganese Chloride, Tetrahydrate, Sigma Prod. No. M-3634.)
- M. Ribonucleic Acid Polymerase Enzyme Solution
(Immediately before use, prepare a solution containing 900 units/ml of Ribonucleic Acid Polymerase in cold Reagent A.)

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PROCEDURE: (continued)

Pipette (in milliliters) the following reagents into suitable 1 ml cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.30	0.30
Reagent C (NTP)	0.05	0.05
Reagent D (DNA Buffer)	-----	0.40
Reagent E (DNA/BSA)	0.40	-----
Reagent F (UDPG/G1,6DP)		0.05
		0.05
Reagent G (β-NADP)	0.05	0.05
Reagent J (PGM/G-6-PDH)		0.01
		0.01
Reagent K (UDPG-PP)	0.01	0.01
Reagent L (MnCl ₂)	0.01	0.01

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent M (Enzyme Solution)	0.10	0.10
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Immediately mix by inversion and record the increase in A_{340nm} for approximately 15 minutes. Obtain the r A_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(10)(0.98)(\text{df})}{(0.00622)(0.1)}$$

10 = Unit definition specifies 10 minute reaction

0.98 = Total volume (in milliliters) of assay

df = Dilution factor

0.00622 = Micromolar extinction coefficient of β-NADPH
at 340 nm

0.1 = Volume (in milliliters) of enzyme used

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

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

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UNIT DEFINITION:

One unit will release 1.0 nanomole (10^{-9} mole) of PP_i in 10 minutes at pH 7.9 at 37°C using calf thymus DNA as template.²

FINAL ASSAY CONCENTRATIONS:

In a 0.98 ml reaction mix, the final concentrations are 82 mM Tris⁶, 31 mM magnesium chloride, 184 mM potassium chloride, 2 mM DL-dithiothreitol, 0.5 mM adenosine 5'-triphosphate, 0.5 mM cytidine 5'-triphosphate, 0.5 mM guanosine 5'-triphosphate, 0.5 mM uridine 5'-triphosphate, 0.04 mM ethylenediaminetetraacetic acid, 0.2% (w/v) bovine serum albumin, 0.02% (w/v) deoxyribonucleic acid, 5 mM uridine 5'-diphosphoglucose, 0.1 mM glucose 1,6-diphosphate, 0.5 mM β -nicotinamide adenine dinucleotide phosphate, 5 units phosphoglucomutase, 5 units glucose-6-phosphate dehydrogenase, 1 unit uridine-5'-diphosphoglucose pyrophosphorylase, 1 mM manganese chloride, 90 units ribonucleic acid polymerase, 1% (v/v) glycerol, and 0.4 mM sodium phosphate.

REFERENCES:

Johnson, J.C., Shanoff, M., Bass, S.T., Boezi, J.A. and Hansen, R.G. (1968) *Anal. Biochem.* **26**, 137-145

Joshi, J.G. (1982) *Methods in Enzymology* **89**, Part D, 599-605

NOTES:

1. This procedure is not to be used to assay the activity of Ribonucleic Acid Polymerase, Sigma Prod. No. R-5376.
2. For the assay of RNA Polymerase from wheat germ, Sigma Prod. No. R-1635, place the DNA solution in a boiling water bath for 10 minutes to denature the DNA. Remove from the water bath and quickly cool in an ice/water bath to below 20°C to obtain single stranded DNA. Do not heat the DNA solution used for assaying the E. coli enzyme. Add the albumin to the solution after the DNA has been heat denatured.
3. Glucose-1,6-Diphosphate is added to ensure that the glucose 1-phosphate continues to glucose 6-phosphate. It is required in order for the phosphoglucomutase to be optimally active.

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NOTES: (continued)

4. Glucose-6-phosphate dehydrogenase is inhibited by ammonium sulfate.
5. The two enzymes listed are ammonium sulfate suspensions.
Add 1000 units of phosphoglucomutase (Sigma Prod. No. P-3397) and glucose-6-phosphate dehydrogenase (Sigma Prod. No. G-7877) to a centrifuge tube. Centrifuge and remove most of the supernatant. Dissolve the pellet in 2 ml of Reagent H (Glycerol Solution) and store at 0 - 5°C. The solution is stable for 1 week. This will give a solution containing 500 units/ml. Sulfate free enzymes of equal specifications can be used without centrifugation; dissolve them in 10 mM sodium phosphate, pH 7.5 at 25°C, containing 50% glycerol.
6. This concentration does not include the concentration of Trizma Base used to adjust the pH of the NTP solution (Reagent C).
7. Phosphoglucomutase Unit Definition: One unit will convert 1.0 μ mole of α -D-glucose 6-phosphate per minute at pH 7.4 at 30°C.
8. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 μ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.
9. Uridine-5'-Diphosphoglucose Pyrophosphorylase Unit Definition: One unit will cause the formation of 1.0 μ mole of glucose 1-phosphate from uridine-5'-diphosphoglucose and inorganic pyrophosphate per minute at pH 7.6 at 25°C.
10. This assay is based on the cited references.
11. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.