Fermentas
LIFE SCIENCES

IMPROVED

CERTIFICATE OF ANALYSIS

Glycogen, molecular biology grade

Inert co-precipitant of nucleic acids.
Derived from oysters.

#R0561 2x0.25ml

Lot:

Concentration: 20mg/ml aqueous solution.

Store at -20°C

In total 2 vials.

Description

The Glycogen is a highly purified polysaccharide derived from oysters. It is an inert carrier, free of host DNA/RNA. The Glycogen is insoluble in ethanol solution; in the presence of salts it forms a precipitate that traps the target nucleic acids. During centrifugation, a visible pellet is formed, which greatly facilitates handling of the target nucleic acids. The Glycogen quantitatively precipitates nucleic acids from diluted solutions with a higher efficiency than that of tRNA, linear polyacrylamide or sonicated DNA (1-4).

Glycogen molecules are highly branched structures composed of thousands of glucose molecules bonded to each other. The molecular weight of the largest individual glycogen molecule containing about 50,000 glucose molecules appears to be 8 million.

Protocol for DNA Precipitation from Diluted Solutions

1. Add 1/10 volume of 3M sodium acetate (or 2M sodium chloride, or 5M ammonium acetate) to DNA solution.
2. Add glycogen to a final 0.05-1µg/µl concentration.
   Use up to 1µl of glycogen per 20µl of the solution. For precipitation of oligonucleotides, maximal 1µg/µl final glycogen concentration is recommended.
3. Add 1 volume of isopropanol (or 2.5 volumes of ethanol) to the solution. Mix gently but thoroughly.
   Ethanol is recommended for precipitation of smaller than 200 bp fragments.
4. Incubate the mixture at -20°C for up to 60min, or at -70°C for 30min.
   Longer incubation at lower temperature provides better recovery of nucleic acids.
5. Centrifuge the mixture for 10-15min at 10,000rpm.
6. Discard the supernatant.
7. Rinse the pellet with cold 70% ethanol.
8. Air-dry the pellet. Avoid over-drying the pellet, as it then takes more time to dissolve.
9. Dissolve DNA in Water, nuclease-free (#R0581) or TE buffer.

Note

- 5µg of Glycogen (0.25µl) forms a clearly visible pellet.
- Optimal for recovery of oligonucleotides (>8 bases) and low amounts (>20pg) of nucleic acids from diluted solutions.
- Up to a final concentration of 8µg/µl, glycogen does not interfere with most downstream applications:
  PCR*, DNA sequencing, DNA digestion by endonucleases, ligation using T4 DNA Ligase (#EL0011), DNA labeling with T4 Polynucleotide Kinase (#EK0031), random priming with DNA polymerases, DNA amplification using phi29 DNA Polymerase (#EP0091).
- Up to 8µg of glycogen does not interfere with bacterial transformation.
- Up to a final concentration of 0.4µg/µl, glycogen does not affect *in vitro* transfection of eukaryotic cells with ExGen 500 *in vitro* Transfection Reagent (#R0511).
- Does not interfere with gel electrophoresis of nucleic acids.
- Does not interfere with spectrophotometrical determination of nucleic acids concentration ($A_{260-280}$ measurements).

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*The Polymerase Chain Reaction (PCR) process is covered by U.S. patents owned by Hoffman-La Roche.

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QUALITY CONTROL ASSAY DATA

Nucleic Acids Precipitation Assay

95% of total radioactivity of 5pg [^{32}P] calf thymus DNA was found in the precipitate after centrifugation. 5pg of [^{32}P] labeled calf thymus DNA (>10^8cpm/µg) were dissolved in 500µl TE buffer with 0.4M LiCl. 1µl glycogen solution (20µg) was added and then precipitated with 1.2ml ethanol at -20°C, stored for 3hrs at -20°C and centrifuged.

Nicking Activity Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 200µg of glycogen with 1µg of pBR322 DNA in 50µl of reaction buffer (33mM Tris-CH_3COOH (pH 7.9), 10mM $Mg(CH_3COO)_2$, 66mM CH_3COOK) for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of 5pmol single-stranded and 5pmol double-stranded [^{32}P] labeled oligonucleotide (17-mer) was observed after incubation with 50µg of glycogen for 16 hours in 10µl of Fermentas Five Buffer System buffers at 37°C and 55°C.

Ribonuclease Assay

No detectable radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 200µg of glycogen with 1µg of *E.coli* [^{3}H]-RNA (40000cpm/µg) in 50µl of reaction buffer (33mM Tris-CH_3COOH (pH 7.9), 10mM $Mg(CH_3COO)_2$, 66mM CH_3COOK) for 4 hours at 37°C.

Protease Assay

No detectable degradation of 0.6% FTC-casein of the Protease Assay Kit (Calbiochem Cat. No. 539125) was observed after incubation with 1000µg of glycogen in 200µl of reaction buffer (200mM Tris-HCl (pH 8.0), 20mM $CaCl_2$, 0.1% NaN_3) for 16 hours at 37°C.

Nucleic Acids Assay

After precipitation of the T4 polynucleotide kinase reaction mixture containing 200µg glycogen and washing with ethanol, radioactivity in the precipitate did not exceed that in the negative control. 200µg glycogen were incubated with 6pmol of [γ-^{32}P]- or [γ-^{33}P]-ATP and 10 units of T4 polynucleotide kinase for 20min in 40µl of reaction buffer (50mM Tris-HCl (pH 7.6), 10mM $MgCl_2$, 5mM DTT, 0.1mM spermidine and 0.1mM EDTA).

Quality authorized by: Jurgita Zilinskiene

References

1. Tracy, S., Improved rapid methodology for the isolation of nucleic acids from agarose gels, Prep. Biochem., 11, 251-268, 1981.
2. Helms, C., A new method for purifying lambda DNA from phage lysates, DNA, 4, 39-49, 1985.
3. Hengen, P. N., Methods and reagents – Carriers for precipitating nucleic acids, TIBS, 21, 224-225, 1996.
4. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the Third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, A8.12-8.13, 2001.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.