

CERTIFICATE OF ANALYSIS

Glycogen, RNA grade

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

#R0551 2x0.1ml

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 RNA Precipitation from Diluted Solutions

- ➊ Add 1/10 volume of 3M sodium acetate (or 2M sodium chloride, or 5M ammonium acetate) to DNA solution.
Use DEPC-treated tubes and tips when working with RNA.
- ➋ 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.
- ➌ 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.
- ➍ 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.
- ➎ Centrifuge the mixture for 10-15min at 10,000rpm.
- ➏ Discard the supernatant.
- ➐ Rinse the pellet with cold 70% ethanol.
- ➑ Air-dry the pellet.
Avoid over-drying the pellet, as it then takes more time to dissolve.
- ➒ Dissolve RNA in DEPC-treated Water (#R0601).

Note

- 5µg of Glycogen (0.25µl) forms a 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:
cDNA synthesis using Reverse Transcriptases (#EP0351, #EP0451, #EP0441), *in vitro* transcription using T7 RNA Polymerase, 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).

*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

1ng of [³³P] labeled RNA (>10⁶cpm/μg) was dissolved in 500μl TE buffer with 0.5M ammonium acetate. To precipitate RNA, a 1μl aliquot of glycogen solution (20μg) and a 1.25ml aliquot of 96% ethanol were added into the mixture. The mixture was stored at -20°C for 15min, and then centrifuged. More than 90% of the initial radioactivity are usually detected in the precipitate of labeled RNA.

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-CH₃COOH (pH 7.9), 10mM Mg(CH₃COO)₂, 66mM CH₃COOK) for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of 5pmol single-stranded and 5pmol double-stranded [³²P] 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* [³H]-RNA (40000cpm/μg) in 50μl of reaction buffer (33mM Tris-CH₃COOH (pH 7.9), 10mM Mg(CH₃COO)₂, 66mM CH₃COOK) 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 CaCl₂, 0.1% NaN₃) 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-³²P]- or [γ-³³P]-ATP and 10 units of T4 polynucleotide kinase for 20min in 40μl of reaction buffer (50mM Tris-HCl (pH 7.6), 10mM MgCl₂, 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.