

Carrier-GLY

For precipitation of small amounts of RNA or DNA
(Catalogue number C083, C084)

rev. 04/2022

Description

Carrier-GLY is a highly purified polysaccharide, glycogen, derived from oysters. It is an inert carrier, free of host DNA, RNA, nickases, DNases and RNases and is preferred over tRNA, yeast RNA or sonicated DNA as a carrier for the precipitation of nucleic acids, because it is less likely to interfere with downstream applications and is more efficient in RNA and DNA precipitation. Low amounts (picograms/ml) of RNA or DNA oligonucleotides as short as 8 base pairs can be recovered using Carrier-GLY at a final concentration 20 µg/ml.

Technical data

Components and packaging

- Carrier-GLY is supplied as a highly purified glycogen in deionized sterile ultrapure water (18 Mohm.cm) at a concentration of ~20 mg/ml.
- Basic packaging is 1 tube containing 1 ml of Carrier-GLY in 2 ml plastic vials with screw cap.
- Carrier-GLY can also be obtained as a part of a set for RNA or DNA precipitations, containing besides Carrier-GLY also Carrier-iRNA and Carrier-ACRYL, 1 ml each. Comparison of various carriers for RNA or DNA precipitation and key references are shown in Table 1.

Storage and Stability

- At temperature $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. Material can be repeatedly defrosted. Unopened vial is stable until the expiration date printed on the label.

Quality control

Each batch of Carrier-GLY is analyzed in several assays. For the assays, DNA or RNA is examined in the Carrier assay buffer (CAB): 10 mM Tris-HCl, 2 mM MgCl₂, 1 mM dithiothreitol, pH 7.5 at 37°C.

- Nucleic acid precipitation assay. Economy DNA marker (2.5 µl; Cat. No. D071) is mixed with 0.2 ml 10 mM Tris, 1 mM EDTA buffer, 1 µl Carrier/GLY, 20 µl of 3 M sodium acetate, pH 5.2, and 0.6 ml of 96% Ethanol. After 30 min at $-20 \pm 5^{\circ}\text{C}$ the mixture is centrifuged at 12,000 x g, analyzed by electrophoresis in agarose gel with ethidium bromide and observed under UV light. More than 90% of all components of the DNA marker is recovered in the precipitate.
- Nick activity assay. Plasmid pUC19 (1 µg) in 200 µl CAB is incubated with Carrier-GLY (50 µg) for 1 hours at 37°C, followed by electrophoresis in agarose gel with ethidium bromide. No nicking activity is observed.
- Ribonuclease assay. RNA (1 µg) in CAB is incubated with Carrier-GLY (50 µg) in 200 µl CAB for 1 hours at 37°C, followed by electrophoresis in agarose gel with ethidium bromide. No changes in properties of RNA are observed under UV light.
- Absence of nucleic acids. Carrier-GLY (200 µg) is loaded on agarose gel with ethidium bromide. After electrophoresis, no bands are observed under UV light.

Cat. No.	Product name and specification	Amount
C083	Carrier-GLY	1 x 1 ml
C084	Carrier-GLY	5 x 1 ml
C085s	Carrier-iRNA+GLY+ACRYL set	1x 0,5 ml + 2x 1 ml



Table 1. Comparison of various carriers for RNA/DNA precipitation.

Carrier	Key component	Advantages	Disadvantages
Carrier-iRNA (previously labeled as RNA carrier)	Polyinosinic acid ⁽¹⁾	Chemically defined RNA, which is more suitable as carrier for cDNA synthesis and other RNA/DNA manipulations than widely used rRNAs or tRNAs..	Inhibits reactions catalyzed by terminal transferase or polynucleotide kinase. Interferes with determination of RNA or DNA concentrations.
Carrier-ACRYL	Linear polyacrylamide ^(2, 3)	Inert neutral carrier, which does not inhibit DNA cloning, DNA-protein interactions, and enzyme reactions. Does not interfere with determination of RNA/DNA concentrations. Does not co-precipitate short oligonucleotides (≤ 20 pbs).	Does not co-precipitate short oligonucleotides (≤ 20 pbs).
Carrier-GLY	Highly purified polysaccharide, glycogen (deprived of RNA, DNA, and proteins) from oysters ⁽⁴⁾	Purified glycogen does not inhibit DNA cloning, and most enzyme reactions and does not interfere with determination of RNA/DNA concentrations. Is suitable as inert carrier for precipitation of shorter oligonucleotides (≥ 8 pbs).	May inhibit some DNA-protein interactions and reverse transcription of long RNA templates.

Reference

1. Winslow, S. G., and P. A. Henkart. 1991. Polyinosinic acid as a carrier in the microscale purification of total RNA. *Nucleic Acids Res.* 19: 3251-3253.
2. Gaillard, C., and F. Strauss. 1990. Ethanol precipitation of DNA with linear polyacrylamide as carrier. *Nucleic Acids Res.* 18: 378.
3. Sachdeva, R., and M. Simm. 2011. Application of linear polyacrylamide coprecipitation of denatured templates for PCR amplification of ultra-rapidly reannealing DNA. *Biotechniques* 50: 217-219.
4. Tracy, S. 1981. Improved rapid methodology for the isolation of nucleic acids from agarose gels. *Prep. Biochem.* 11: 251-268.

Protokol

Equipment and reagents required but not provided

- Microcentrifuge (12.000 x g)
- PCR Sodium acetate buffer solution, 3 M, pH 5.2. Top-Bio, Cat. No. P053
- PCR Ethanol, 96%, mol. biol. grade, Top-Bio, Cat. No. P054
- 10 mM Tris - 1 mM EDTA buffer, prepared by 100x dilution from Tris-EDTA buffer solution , Top-Bio, Cat. No. P055
- PCR Ultra H2O (Top-Bio Cat. No. P040)

Procedure

1. To a maximum of 400 μ l of RNA or DNA sample in a microcentrifuge tube add 1 μ l of Carrier-GLY (corresponding to 20 μ g of glycogen).
2. Add 0.1 volume of 3M sodium acetate, pH 5.2.
3. Add 2.5 – 3.0 x sample volume of 96% ethanol.

Example reagent volumes

DNA/RNA sample solution	Carrier-GLY	3M Sodium acetate buffer	Ethanol 96%
200 μ l	1 μ l	20 μ l	600 μ l

4. Vortex the mixture for 2 seconds and allow to stand for at least 30 min at -20oC.
5. Centrifuge the tubes for 15 minutes at 4°C in a microcentrifuge at maximum speed (12,000 x g) .
6. Carefully remove the supernatant and add to the pellet 70% ethanol.
7. Centrifuge for 2 minutes and carefully remove supernatant

8. Air-dry the pellet for 15-30 min. Avoid over-drying as it then takes more time to dissolve.
9. Dissolve RNA or DNA in 10 mM Tris-1 mM EDTA buffer, PCR ultra H₂O or PCR H₂O.