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Carrier-iRNA

For precipitation of small amounts of RNA or DNA (Catalogue number C078, C079)

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Description

Carrier-iRNA is polyinosinic acid of Molecular Biology Grade. It is an efficient inert carrier for ethanol precipitation of picograms and higher quantities of RNA or DNA. Carrier-iRNA offers several advantages over other carriers, such as tRNA, yeast RNA, or sonicated DNA, for recovering nucleic acids prior to downstream applications. Carrier-iRNA is synthetic polymer, which is not source of biological contamination in the samples. Nucleic acids recovered after precipitation in the presence of Carrier-iRNA are immediately suitable for downstream applications such as PCR and RT-PCR.

Technical data

Components and packaging

- Carrier-iRNA is supplied in deionized, ultrapure, and sterile water (18 Mohm.cm) at a concentration of ~10 mg/ml.
- Basic packaging contains 0,5 ml of Carrier-iRNA in 2 ml plastic vials with screw cap.
- Carrier-iRNA is a part of the set for RNA or DNA precipitations, containing besides Carrier-iRNA also Carrier-ACRYL and Carrier-GLY, 1 ml each. Comparison of various carriers for RNA or DNA precipitation and key references are shown in Table 1.

Storage and Stability

• Store at temperature -20°C ± 5°C. Carrier-iRNA is stable until the expiration date printed on the tube label. To reduce the viscosity after freezing, we recommend heating the Carrier-iRNA tube to 37°C for 15 min.

Quality control

Each batch of Carrier-iRNA 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 (Cat. No. D071; 2.5 μl) is mixed with 0.2 ml 10 mM Tris buffer, pH 8.0 + 1 mM EDTA, 1 μl Carrier-iRNA, 20 μl of 3 M sodium acetate, pH 5.2, and 0.6 ml of 96% Ethanol. After 30 minutes at 2 8°C the mixture is centrifuged for 10 min 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 μ g) in 0.2 ml CAB is incubated with Carrier-iRNA (50 μ g) for 1 hours at 37°C, followed by electrophoresis in agarose gel with ethidium bromide. No nicking activity is observed.
- Ribonuclease assay. RNA (1 μ g) in 50 μ l CAB with Carrier-iRNA (50 μ g) is incubated 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.

Cat. No.	Product name and specification	Amount
C078	Carrier-iRNA	1 x 0,5 ml
C079	Carrier-iRNA	5 x 0,5 ml



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

Carrier	Key component	Advantages	Disadvantages
Carrier-iRNA	Polyinosinic acid (1)	Chemically defined RNA, which is	Could inhibit reactions catalyzed
(previously labeled as		more suitable as carrier for cDNA	by terminal transferase or
RNA carrier)		synthesis and other RNA/DNA	polynucleotide kinase. Interferes
		manipulations than widely used	with determination of RNA or
		rRNAs or tRNAs.	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).	
Carrier-GLY	Highly purified polysaccharide, glycogen (deprived of RNA and DNA) from oysters ⁽⁴⁾	Purified glycogen does not inhibit DNA cloning and most enzyme reactions; does not interfere with determination of RNA/DNA concentrations. It 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.

Protocol

Equipment and reagents required but not provided

- Microcentrifuge (12.000 x g)
- Sodium acetate buffer solution, 3 M, pH 5.2. Top-Bio, Cat. No. P053
- Ethanol, 96%, Mol. Biol. Grade, Top-Bio, Cat. No. P054
- Ethanol, 75%, Mol. Biol. Grade, Top-Bio Cat. No. P044
- 10 mM Tris-HCl + 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) or PCR H₂O (Top-Bio, Cat. No. P442)

Procedure

- 1. To a maximum of 400 μ l of RNA or DNA sample in a 1.5 ml tube add 1 μ l of Carrier-iRNA (10 μ g).
- 2. Add 0.1 volume of 3 M sodium acetate, pH 5.2.
- 3. Add 2.5 3.0 x sample volume of 96% ethanol.

Example reagent volumes

DNA/RNA sample	Carrier-iRNA	3M Sodium acetate buffer	Ethanol 96%
200 μΙ	1 μΙ	20 μΙ	600 μΙ

- 4. Vortex the mixture briefly (2 sec) and allow to stand for at least 30 min at 2 8°C.
- 5. Centrifuge the tubes for 15 min at 4°C in a microcentrifuge at maximum speed (12,000 x g).
- 6. Carefully remove the supernatant and add 200 μl 75% ethanol.
- 7. Centrifuge for 2 min and carefully remove supernatant.
- 8. Air-dry the pellet for 15 min.
- 9. Dissolve RNA or DNA in 10 mM Tris-HCl + 1 mM EDTA buffer, PCR ultra H_2O , or PCR H_2O .