

Carrier-iRNA

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

rev. 11/2022

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 $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. 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 MgCl_2 , 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 $2 - 8^{\circ}\text{C}$ the mixture is centrifuged for 10 min at $12,000 \times g$, 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 0.2 ml CAB is incubated with Carrier-iRNA (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 50 μl CAB with Carrier-iRNA (50 μg) 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
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.	Could inhibit 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 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 μ l	1 μ l	20 μ l	600 μ l

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 μ l 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 H₂O, or PCR H₂O.