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Carrier-ACRYL

For precipitation of small amounts of RNA or DNA
(Catalogue number C081 and C082)

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Description

Carrier-ACRYL is linear polyacrylamide (LPA) of Molecular Biology Grade. It is an efficient inert carrier for ethanol precipitation of picograms and higher quantities of RNA or DNA. Carrier-ACRYL offers several advantages over other carriers, such as tRNA, yeast RNA, or sonicated DNA, for recovering nucleic acids prior to downstream applications. Carrier-ACRYL is synthetic polymer, which is not source of biological contamination in the samples. Presence of Carrier-ACRYL during ethanol precipitation results in complete recovery of fragments larger than 20 base pairs while failing to precipitate shorter fragments and free nucleotides. This feature makes Carrier-ACRYL useful for separating reactions products from unincorporated nucleotides and oligonucleotide primers. Nucleic acids recovered after precipitation in the presence of Carrier-ACRYL are immediately suitable for downstream applications such as PCR, RT-PCR, restriction digestion, ligation, and sequencing.

Technical data

Components and packaging

- Carrier-ACRYL is supplied in deionized, ultrapure, and sterile water (18 Mohm.cm) at a concentration of ~25 mg/ml.
- Basic packaging contains 1 ml of Carrier-ACRYL in 2 ml plastic vials with screw cap.
- Carrier-ACRYL is a part of the set for RNA or DNA precipitations, containing besides Carrier-ACRYL also Carrier-iRNA 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

- For short terms (months) store at temperature 4°C ± 3°C.
- For long terms store at temperature -20°C ± 5°C.

Quality control

Each batch of Carrier-ACRYL 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-ACRYL, 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 µg) in 0.2 ml CAB is incubated with Carrier-ACRYL (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-ACRYL (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
C081	Carrier-ACRYL	1 x 1 ml
C082	Carrier-ACRYL	5 x 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 H_2O (Top-Bio Cat. No. P040) or PCR H_2O (Top-Bio, Cat. No. P442)

Procedure

1. To a maximum of 400 µl of RNA or DNA sample in a 1.5 ml tube add 1 µl of Carrier-ACRYL (corresponding to 25 µg of Carrier-ACRYL).
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-ACRYL	3M Sodium acetate buffer	Ethanol 96%
200 µl	1 µl	20 µl	600 µl

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.