ExpressArt RNA Clean RNAready

RNA Clean-up kit

Catalogue No. 9003-A100

for 100 RNA Clean-up procedures

Purification of in vitro transcription products,
Removal of small RNAs (below ~200nt) from RNA samples,
Dye removal from labelled RNA

This protocol provides the required laboratory procedures

For additional information about ExpressArt technology and products see
http://www.amp-tec.com/products
» more information

Kit Contents

	Volume per RNA isolation	Kit contents for 100 isolations
Lysis Buffer (LB)	**340 µl**	**40 ml**
DeS Buffer	**87 µl**	**10 ml**
Wash Buffer 1 (WB 1): add 18.0 ml ethanol (100%) to 36.0 ml concentrate of WB 1	**500 µl**	**36 ml concentrate**
Wash Buffer (WB 2): add 44.0 ml ethanol (100%) to 11.0 ml concentrate of WB 2	**500 µl**	**11 ml concentrate**
RNase-free water	**30 µl**	**5 ml**
Spin columns with collection tubes	**1 piece**	**100 pieces**

Please note, these reagents are not included in the kit
Ethanol, abs.

Chemical hazards

The Lysis Buffer (LB) contains guanidine thiocyanate, which is harmful in contact with skin, when inhaled or swallowed. Guanidine thiocyanate also liberates toxic gas, when mixed with strong acids. Always store and use the Binding Buffer away from food. Always wear gloves, and follow standard safety precautions during handling and make sure to comply with the safety rules of your laboratory.

PROCEDURE

Purification of in vitro transcription products, removal of small RNAs (below ~200nt) from RNA samples, dye removal from labelled RNA, etc., with Spin Columns

Before starting: Add **18 ml of 100% ethanol to WB1 concentrate (36 ml)** and **44 ml of 100% ethanol to WB2 concentrate (11 ml)**, as indicated on the bottles.

At the start: Put **RNase-free water** in thermoblock at 95℃.

RNA Purification Mix	
LB Buffer	340 µl
DeS Buffer	87 µl
100% Ethanol (at room temperature; not supplied)	560 µl

- Add 987 µl of **RNA Purification Mix** to 20 µl RNA sample (adjust smaller volumes by addition of water). Mix thoroughly.
- Transfer an aliquot of **700 µl of this mixture** on the RNA spin column, centrifuge for 30 sec@10'000 rpm & discard flow-through
- Transfer the **remaining mixture** on column, centrifuge for 30 sec@10'000 rpm & discard flow-through
- Add 500 µl **Wash Buffer 1 (WB 1)**, centrifuge for 30 sec@10'000 rpm & discard flow-through
- Add 500 µl **Wash Buffer 2 (WB 2)**, centrifuge for 30 sec@10'000 rpm & discard flow-through
- Re-insert the column in the same collection tube. Add 500 µl **80% EtOH,** centrifuge for 30 sec@10'000 rpm & discard flow-through
- Re-insert the column in the same collection tube, and centrifuge for 1 min@maximum speed to get rid of residual salt on the spin column matrix.
- **Elution**: Transfer column in fresh 1.5 ml reaction tube and add 30 µl **RNase-free water** (preheated at 95℃)
- Incubate: 2 min and centrifuge for 1 min@maximum speed
- Reapply the eluate on the column
- Incubate: 2 min and centrifuge for 1 min@maximum speed
- RNA is in a total volume of ~ 30 µl.
- Use immediately or store at -80℃.
- **Long-term storage or shipping:**
  a) for concentrations ≥1 µg/µl, storage at -80℃ and shipping on dry ice is adequate
  b) for low concentrations, storage/shipment as EtOH suspension is recommended:
     add 1/10 volume of sodium acetate (pH 5) and 1 to 2 µl of carrier, like "Pellet Paint" (Novagen), mix thoroughly; then add 2.2 volumes of EtOH (100%).
     Store at -20℃ / ship on dry ice.
     RNA is recovered by centrifugation (20min@max speed, at 4℃), remove supernatant with a pipette, wash with 200 µl 80% EtOH, spin again, discard supernatant and air dry the RNA pellet, dissolve in RNase-free water.

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