EURx® MOLECULAR BIOLOGY PRODUCTS

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Universal RNA Clean-Up Kit

Universal kit for DNA digestion in RNA samples

Cat. no. E3589

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Table of Contents

Content	25 preps E3589-01	100 preps E3589-02	Storage/Stability
DNR II	1.5 ml	6 ml	2-8°C
DNase I (5 U / 1 µl)	275 U	4 x 275 U	-20°C
DNase I buffer	100 µl	300 µl	15-25°C
RL	12 ml	48 ml	15-25°C
Wash RNA	27 ml	108 ml	15-25°C
RNase-free water	3 ml	12 ml	15-25°C
RNA Binding Columns	25 szt.	2 x 50 szt	2-8°C
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Introductory Notes

NOTE 1 · Kit Specification. This kit is designed for total RNA clean up from the remaining DNA and other contaminants with the use of DNase I and selective spin columns. Kit can be used for previously purified RNA samples from a variety of sources.

NOTE 2 · Maximum Column Binding Capacity. The total RNA binding capacity is 100 µg. The maximum volume of the column reservoir is 600 µl. Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

NOTE 3 · Kit Compounds Storage. Keep all solutions tightly closed to avoid evaporation, resulting in components concentration changes. Upon arrival store RNA Binding Columns and DNR II in 2-8°C. Freeze-dried/resuspended DNase I at -20°C. Other components keep at room temperature.

NOTE 4 · β Mercaptoethanol / DTT. Contaminating RNases are inactivated by addition of reducing agents capable of disrupting disulfide bonds, such as ß-mercaptoethanol (ß-ME) or dithiothreitol (DTT). To promote reduction of disulfide bonds, add 10 µl ß-ME per 1 ml of buffer RL before use. Upon addition of ß-ME, RL buffer remains stable for 1 month. A less toxic but more expensive alternative to ß-ME is, to add 10 µl of [1 M] DTT in RNase free water per 1 ml buffer RL buffer before use. DTT is not stable in RL buffer, thus DTT-supplemented RL buffer aliquots must not be stored. Working aliquots of [1 M] DTT stock solution in RNase free water must be stored at -20°C for maintaining stability. To set up a [1 M] DTT stock solution (MW = 154.25 g mol-1), dissolve 1.54 g DTT in 10 ml RNase free water and store in aliquots for one-time usage.

NOTE 5 · DNase I treatment should be performed with DNR II reaction buffer. Do not use other reaction buffers.

NOTE 6 · Before starting RNA clean up, dissolve DNase I in DNase I buffer according to the instructions provided in **Part I Before starting** Step 1.

NOTE 7 · Maintaining Good Working Practice. To obtain high quality RNA, stick carefully to the protocol provided below. One of the most critical issues during RNA clean up is, to ensure working quickly and with practiced hand. RNA clean up should be performed at room temperature throughout the entire process. Avoid introducing any RNases during the procedure or later handling.

Equipment and reagents to be supplied by user

- Ethyl alcohol [96-100% v/v], β-merkaptoethanol (14.3 M, β-ME) or 1 M dithiothreitol (DTT) in RNase-free water, microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5–2 ml tubes.

Protocol

Part I Before starting

1. Dissolve freeze-dried **DNase I** by adding **DNase I buffer** to obtain the concentration of 5U / µl.

   - *25 preps kit (EE3589-01) contains 1 tube with freeze-dried 275 U of DNase I, that should be dissolved in 55 µl of DNase I buffer.*

   - *100 preps kit (EE3589-02) contains 4 tubes, each with 275 U of DNase I. Add 55 µl of DNase I buffer into each tube in order to dissolve DNase I enzyme.*

   - *DNase I is sensitive to physical denaturation. Therefore, do not vortex solutions containing DNase I, mix by rotating the tube or pipetting.*

   - *Add DNase I buffer to the tube containing freeze-dried DNase I, wait 3 min and gently mix by pipetting. Store the enzyme in temp. -20°C.*

2. Equilibrate samples to room temperature.

3. Make sure that β-merkaptoethanol is added to **RL** buffer (see Note 4 page 3)

Part II RNA/miRNA clean up

1. To 80 µl RNA/miRNA add 20 µl of **DNR II** buffer, mix by pipeting and add 2 µl of **DNase I**. Mix gently by pipeting.

   - *RNA/miRNA volume can be scaled up (or down) in the proportion 4 to 1 DNR II buffer. For each 100 µl of the obtained mixture add 2 µl DNase I.*

2. Incubate for 20 min in room temperature (15-25°C) with gentle shaking.

   - *DNase I digestion effectively removes DNA from the sample.*

3. After 20 min add 3 volumes of **RL** buffer to 1 volume of the sample and afterwards 1.2 volumes of **96% ethyl alcohol** and mix.

   - *e.g. to 100 µl of RNA add 300 µl of RL buffer and 480 µl of ethyl alcohol.*

4. Carefully transfer 600 µl of the solution (with any precipitate if forms) to **RNA binding column** in 2 ml receiver tube. Close the cap and centrifuge at 11 000 x g for 1 min.

5. Remove the spin column, pour off supernatant and place back into the receiver tube.

6. Transfer the remaining mixture to the same **RNA binding column** and centrifuge for 1 min at 11 000 x g.

7. Remove the spin column, pour off supernatant and place back into the receiver tube.

8. Add 600 µl **Wash RNA** buffer. Close the cap and centrifuge at 11 000 x g for 1 min.

9. Remove the spin column, pour off supernatant and place back into the receiver tube.

10. Add 300 µl **Wash RNA** buffer. Close the cap and centrifuge at 11 000 x g for 2 min.

    - *Be careful not to contaminate the sample while removing the spin-column from the receiver tube. Check, whether the membrane of the spin column is completely dry. If not, pour off any remaining supernatant and place back spin-column into the receiver tube. Spin down for one additional min.*

11. Place spin-column in a clean microcentrifuge tube (1.5-2 ml) and add 50-100 µl **RNase-free water** directly onto the membrane.

12. Incubate spin-column for 1 min at room temperature and centrifuge at 11 000 x g.

13. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. RNA can be stored either at 2-8°C (preferred) or at -20°C. Avoid multiple freezing and thawing.

Safety Information

RL

Danger

H302+**H332** Harmful if swallowed or if inhaled.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
P301+**P312** If swallowed: call a poison center/ doctor/... if you feel unwell.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
EUH032 Contact with acids liberates very toxic gas

Wash RNA

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+**P235** Store in a well-ventilated place. Keep cool.
P337+**P313** If eye irritation persists: get medical advice/ attention

Universal RNA Clean-Up Kit removes DNA and other contaminants from RNA samples isolated from different sources (animal and plant tissues, bacteria, yeast, blood etc.).

RNA can be purified by phenol-chloroform extraction, spin column or the use of magnetic beads. Nevertheless it is usually contaminated by genomic DNA which affects downstream applications like RT-qPCR or RNAseq. In the first step DNase I and a reaction buffer are added to the RNA sample in order to digest DNA. Then the nucleic acid binding buffer is added to the sample. This buffer inactivates any RNases/DNases present in the sample. During short centrifugation RNA binds selectively to the membrane. Unbound impurities remain in the column flow-through. Traces of contaminants remaining on the membrane are efficiently removed in two wash steps.

The elution of purified RNA is carried out with RNase-free water. Purified nucleic acids have a length of over 25 nt (miRNA) and are free of proteins, nucleases, other impurities and are ready for use in amplification reactions or storage at -20°C.

The use of unique chemical composition of the matrices, in combination with optimized spin-column design and buffers enables the RNA recovery up to 100 %.

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