TIANGEN®

RT240726

RNase-Free DNase I

Cat. no. GRT411

Kit Contents

components	GRT411 50 preps
RNase-Free DNase I (lyophilized)	1500 U
Buffer RDD (DNA Digest Buffer)	4 ml
RNase-Free ddH_2O	1 ml

Storage

The RNase-Free DNase I is shipped at room temperature (15-30°C). Stored at 2-8°C for 15 months.

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Important Notes

1. In some cases, the vial of DNase I may appear to be empty. This is due to lyophilized enzyme sticking to the septum. To avoid loss of DNase I, do not open the vial. Instead, inject RNase-free water into the vial using a needle and syringe, invert the vial to dissolve the DNase I, and remove the dissolved DNase I using the syringe and needle.
2. Insoluble material may remain when dissolving DNase I. This does not affect DNase I performance. Due to the production process, insoluble material may be present in the lyophilized DNase I.
3. DNase I is sensitive to physical denaturation. Mix gently by inverting the tube. Do not vortex.
4. The RNase-Free DNase I could effectively eliminate DNA, and compatible with TIANGEN RNAprep Pure and RNA Easy Fast series kits.
5. The RNase-Free DNase I provides efficient on-column digestion of DNA during RNA purification using with TIANGEN RNA kits, and also could be used in DNA digestion in RNA solution.

Protocol

A. Used with TIANGEN RNA kits (on-column)

Buffer RDD is optimized for on-column DNase I digestion. (Below protocol is used along with RNAprep Pure kits, RW1 is provided by RNAprep Pure kits).

Note: Ordinary DNase Buffer probably not suits for on-column DNA digestion, other buffer also may affect the combination of RNA and membrane, which lead to low RNA yield.

1. Preparation of DNase I stock solution: Dissolve the lyophilized DNase I (1500 units) in 550 µl of the RNase-free ddH_2O. Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months. Thawed aliquots can be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
2. Preparation of DNase I working solution: Add 10 µl DNase I stock solution (see Preparation of DNase I stock solution) to 70 µl Buffer RDD. Mix by gently inverting the tube
3. Continue with the protocol in RNAprep Pure kit. Add 350 µl Buffer RW1 to the RNA adsorption column and centrifuged at 12,000 rpm (~ 13,400×g) for 30-60 sec. Discard the waste liquid.
4. Add 80 µl DNase I working solution prepared in step 2 to the center of the adsorption column, and place it at room temperature for 15 min.
5. Add 350 µl Buffer RW1 to the column and centrifuge for 30-60 sec at 12,000 rpm (~13,400×g), discard the flow through. Continue to follow the RNAprep Pure kit procedure until the final elution of RNA is achieved.

Note: For further experiments, please follow the RNAprep Pure Series instructions.

B. Directly prepare RNA solution

1. Preparation of DNase I stock solution: Dissolve the lyophilized DNase I (1500 units) in 550 µl of the RNase-free ddH_2O. Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months. Thawed aliquots can be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
2. Set up the reaction system in a RNase-free centrifuge tube:

   ≤87.5 µl of RNA solution
   10 µl of Buffer RDD
   2.5 µl of DNase I stock solution
   Up to 100 µl with RNase-Free H_2O
3. Incubate at 20-25°C for 10 min
4. RNA purification, use TIANGEN RNAclean Kit (GDP412)

   Note: The introduction of DNase I and different concentrations of salt ions into the processed RNA may affect the subsequent experiments. In order to ensure the smooth operation of the next step, it is recommended to use the column purification method to purify the RNA. If only a simple inactivation treatment is needed for DNase I, 10 µl stop solution (20 mM EDTA, pH8.0) can be added to the system after the treatment in step 3, and DNase I can be inactivated after incubation at 65°C for 10 min.

C. Used with TIANGEN Magnetic beads RNA extraction kits

Note: Please refer to the manuals of corresponding magnetic beads RNA extraction kits for the DNase I digestion system.