DANAGENE BACTERIA RNA KIT

Ref.0804.1 100 preps
Ref.0804.2 500 preps

1. INTRODUCTION

DANAGENE BACTERIA RNA Kit allows the extraction of **total RNA** from different bacterial and yeast cultures avoiding the use of toxic reagents.

The process includes a cell lysis followed by the precipitation of the proteins and part of the genomic DNA. Later, by a precipitation with isopropanol, the total RNA is obtained, which is finally hydrated.

Gram (+) strains and yeast are more difficult to lysate than Gram (-) strains and for this reason it´s necessary the preincubation with lytic enzymes (not supplied with this kit. In case of Gram (+) strains, lysozyme is used, and zymolyase or lyticase is used for yeast.

Características:

- **Fast and easy method for an efficient total RNA purifiфcation form bacteria or yeast.**
- **Safe method, as NO TOXIC reagents are used.**
- **It can process 100 bacteria o yeast samples of 1ml.**

2. KIT COMPONENTS

	100 preps	500 preps	Stock
Lysis Solution	**60 ml**	**300 ml**	**RT**
Protein Precipitation Solution	**30 ml**	**150 ml**	**RT**
Hydration Solution	**10 ml**	**50 ml**	**RT**

2.1Equipment and additional reagents required

- Microcentrifuge.
- Microtubes of 1. 5 ml and 2.0 ml.
- Ethanol 70%.
- Isopropanol.
- Lysozyme/ Lyticase or zymolyase.
- Water bath, dry bath or block with heating (90ºC).

3. PROTOCOL

3.1 General Remarks

RNases exist in prokaryotes as well as in eukaryotes, and almost in every cell type. The main RNases sources are microorganisms (bacteria, fungi and their spores) and their products (restriction enzymes, polymerases, etc.). For such reason, they can be present in a Molecular Biology laboratory in many places and it is convenient to be careful with the following recommendations:

1. Use gloves to prevent contamination by RNases present in hands.
2. Change frequently your gloves.
3. Have pipettes for working exclusively with RNA.
4. Use RNase-free tubes and tips.
5. Use RNase-free reagents.
6. Have a determined area in the lab to work with RNA.

3.2 Protocol for total ARN isolation from bacterial cultures of 1ml Gram(-) o Gram(+)

Gram (+) strains are more difficult to lyse than Gram (-) strains, for this reason it is recommended to do an incubation with lytic enzymes. For certain species of Staphylococcus it is necessary a lysis with a mixture of lysozyme (10 mg/ml) and lysostaphin (10 mg/ml).

1. Add 1ml of an overnight culture to a 1.5 ml. microtube.
2. Centrifuge at 14.000 xg for 30 seconds. Remove the supernatant. **For Gram (+) strains proceed with step 3. For Gram (-) strains go directly to step 6.**
3. Resuspend the cells in 540 µl of nuclease-free water or EDTA 50 mM.
4. Add 60 µl of Lisozyme (10 mg/ml). The purpose of this treatment is to weak the cell wall to make the cell lysis more efficient.
5. Incubate the sample at 37ºC for 60 minutes. Invert the periodically the sample during the incubation. Centrifuge at 14.000 xg for 2 minutes. Remove the supernatant.
6. Add 600 µl of **Lysis Solution** to the cell pellet. Use a micropipette to resuspend and to lyse the cells.
7. Incubate the sample at 65ºC for 5 minutes. Allow to air dry.
8. Add 300 µl of **Protein Precipitation Solution.** Invert the tube about 8-10 times and incubate in ice for 5 minutes.
9. Centrifuge at 14.000 xg for 5 minutes. The protein precipitate will form a tight pellet.
10. Pour the supernatant containing the RNA in a new microtube with 600 µl of **Isopropanol.** Mix by inversion for several times.
11. Centrifuge at 14.000 xg for 3 minutes.
12. Remove the supernatant. Add 600 µl of **Ethanol 70 %** and invert the tube several times to wash the RNA pellet.
13. Centrifuge at 14.000 xg for 2 minutes. Carefully, avoiding losing the pellet. Remove all the ethanol.
14. Invert and drain the tube on absorbent paper and allow to air dry for 15 minutes.
15. Add 100 µl of **Hydration Solution.** Use the micropipette for resuspending the pellet.
16. Allow the RNA to hydrate on ice for at least 30 minutes. Use the micropipette for resuspending the pellet.
17. Store the samples at −70ºC.

If the extracted RNA will be used in RT-PCR, to remove the contaminating DNA use our DANAGENE DNA Removal Kit

3.3 Protocol for total RNA isolation from yeast cultures of 1ml

1. Add 1ml of an overnight culture to a 1.5 ml. microtube.
2. Centrifuge at 14.000 xg for 2 minutes. Remove the supernatant.
3. Resuspend the cells in 592μl of nuclease-free water or EDTA 50 mM.
4. Add 8 μl of Lyticase or Zymolyase (20 mg/ml). The purpose of this treatment is to weak the cell wall to make the lysis more efficient.
5. Incubate the samples at 37ºC for 60 minutes. Invert periodically the sample during the incubation. Centrifuge at 14.000 x g for 2 minutes. Remove the supernatant.
6. Add 600 μl of **Lysis Solution** to the cell pellet. Use a micropipette to resuspend and to lyse the cells.
7. Incubate the sample at 65ºC for 5 minutes. Allow to air dry.
8. Add 300 μl of **Protein Precipitation Solution.** Invert the tube for 8-10 times and incubate in ice for 5 minutes.
9. Centrifuge at 14.000 x g for 5 minutes. The protein precipitate will form a tight pellet.
10. Pour the supernatant containing the RNA in a new microtube containing 600 μl of **Isopropanol.** Mix by inversion several times.
11. Centrifuge at 14.000 x g during 3 minutes.
12. Remove the supernatant. Add 600 μl of **Ethanol 70 %** and invert the tube several times to wash the RNA pellet.
13. Centrifuge at 14.000 x g for 2 minutes. Carefully, avoiding to lose the pellet, remove all the ethanol.
14. Invert and drain the tube on absorbent paper to allow to air dry for 15 minutes.
15. Add 100 μl of **Hydration Solution.** Use a micropipette for resuspending the pellet.
16. Allow the RNA to hydrate on ice for at least 30 minutes. Use a micropipette for resuspending the pellet.
17. Store the samples at –70ºC.

If the extracted RNA will be used in RT-PCR, to remove the contaminating DNA use our DANAGENE DNA Removal Kit

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es