
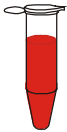


## innuPREP RNA Mini Kit 2.0

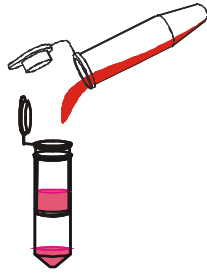
### Protocol 3: RNA extraction from bacterial cells

Recommended steps  
before starting

- Prepare Washing Solution HS, Washing Solution LS according to the instruction

|   |  |   |
|---|--|---|
| 1. Starting material                              | Bacterial cells  | <ul style="list-style-type: none"> <li>▪ Max. <math>1 \times 10^9</math> cells</li> </ul>   |
| 2. Pellet cells                                   |   | <ul style="list-style-type: none"> <li>▪ 5,000 x g (~7,500 rpm); 5 min</li> <li>▪ Discard supernatant</li> </ul>  |
| 3. Pre-lysis                                      |  | <ul style="list-style-type: none"> <li>▪ Add 100 µl TE-Buffer</li> <li>▪ Resuspend cell pellet</li> </ul>   |
|   | Gram-negative bacteria   | <ul style="list-style-type: none"> <li>▪ Add 20 µl Lysozyme</li> <li>▪ Incubation until clear and viscous solution</li> </ul>   |
|   | Gram-positive bacteria   | <ul style="list-style-type: none"> <li>▪ Add 20 µl Lysozyme</li> <li>▪ Add 5 µl Mutanolysin</li> <li>▪ Place on a shaking platform</li> <li>▪ Incubation: 30 min @ 37 °C</li> </ul> |
|   | Staphylococcus strains   | <ul style="list-style-type: none"> <li>▪ Add 20 µl Lysostaphin</li> <li>▪ Place on a shaking platform</li> <li>▪ Incubation: 30 min @ 37 °C</li> </ul>                              |
| 3. Pre-lysis with innuPREP Bacteria Lysis Booster | optional   | <ul style="list-style-type: none"> <li>▪ Add 170 µl TE-Buffer</li> <li>▪ Resuspend cell pellet</li> <li>▪ Add 20 µl Enzyme Mix</li> <li>▪ Incubation: 30 min @ 37 °C</li> </ul>     |
| 4. Lysis  |  | <ul style="list-style-type: none"> <li>▪ Add 450 µl RL</li> <li>▪ Incubation: 3 min @ RT</li> </ul>   |

## 5. Selective removing of gDNA



- Spin Filter D to Receiver Tube
- Add lysed sample to Spin Filter D
- 11,000 x g (~11,000 rpm): 2 min
- Discard Spin Filter D
- Add equal volume 70 % ethanol (approx. 600 µl) to filtrate

## 6. Selective binding of RNA

New Receiver Tube



- Spin Filter R to Receiver Tube
- 650 µl sample to Spin Filter R
- 11,000 x g (~11,000 rpm): 1 min
- Add residual sample
- 11,000 x g (~11,000 rpm): 1 min

## 7. Washing

New Receiver Tube



- Add 500 µl Washing Solution HS
- 11,000 x g (~11,000 rpm): 1 min
- Add 700 µl Washing Solution LS
- 11,000 x g (~11,000 rpm): 1 min

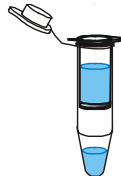
## 8. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Spin Filter R to Receiver Tube
- 11,000 x g (~11,000 rpm): 3 min

## 9. Elution



- Spin Filter R to an Elution Tube
- Add 30–80 µl RNase-free water
- Incubation: 1 min @ RT
- 11,000 x g (~11,000 rpm): 1 min

|                   |                |               |
|-------------------|----------------|---------------|
| <b>Order No.:</b> | 845-KS-2040010 | 10 reactions  |
|                   | 845-KS-2040050 | 50 reactions  |
|                   | 845-KS-2040250 | 250 reactions |

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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