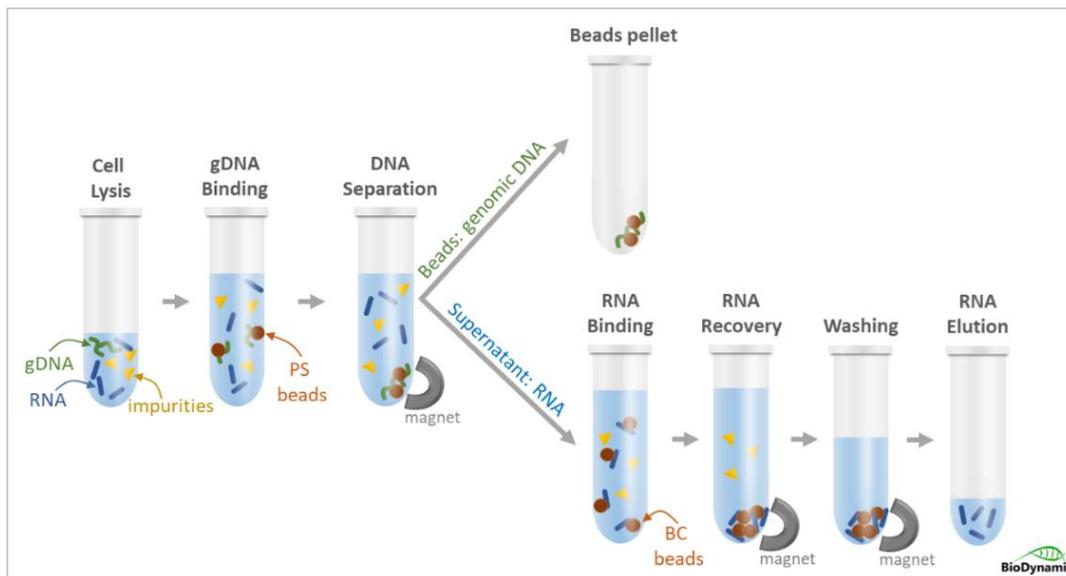


Bacterial RNA Extraction Kit (Magnetic Beads)

Catalog No.	50301S	50301L
Preps	10 preps	50 preps

Description

The **Bacterial RNA Extraction Kit (Magnetic Beads)** enables efficient microbial RNA isolation from cultures of gram-negative and gram-positive bacteria, yeast, and fungi using magnetic bead technology. The kit provides a reliable and straightforward approach for obtaining intact bacterial and microbial RNA with high-yield recovery and fast magnetic response time.



The RNA extraction procedure is simple and efficient: samples are lysed using a buffer and bead mixture; beads binding to genomic DNA are discarded, and the supernatant containing RNA is mixed with a second beads for purification and elution. The kit allows multiple samples to be processed simultaneously and is free of toxic substances such as phenol and chloroform.

The purified total RNA is of high quality and integrity, free from contamination by genomic DNA, salts, proteins, and other impurities. RNA extracted using this kit is suitable for various downstream applications, including qPCR, RT-PCR, hybridization, and next-generation sequencing (NGS).

Features

- High Quality
 - Extract intact total RNA
 - Free of contaminations and impurities
- No additional DNase treatment needed.
- Safe procedure
 - No phenol
 - No chloroform
- Simple
 - No column needed
 - No vacuum needed

Component

Catalog No.	50301S	50301L
Resuspension Buffer (containing RNase inhibitor)	2 ml	10 ml
PS Beads	1 ml	5 ml
KG Buffer	1 ml	5 ml
BC Beads	2.2 ml	11 ml
Nuclease-free Water	0.5 ml	2.5 ml

Storage Condition

- Store Resuspension Buffer, BC Beads at 4°C, stable up to 12 months.
- Store PS Beads, KG Buffer, and Nuclease-free Water at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- Vortexer
- Water bath or heat block
- 1.5 ml or 2.0 ml tubes
- 80% ethanol (prepare before use)
- Lysozyme (20 mg/ml) for samples of gram-positive bacteria or unknown strains.
- Lysostaphin (2 mg/ml) for samples of lysozyme-resistant gram-positive bacteria.

Protocol

1. Transfer bacteria culture (up to 10^9 bacteria cells are OK) to 2 ml tubes and centrifuge at 5000 g for 5 min. Remove supernatant and resuspend the pellet in 200 μ l of **Resuspension Buffer** (containing RNase inhibitor).

Note:

- Add 180 μ l of **Resuspension Buffer** and 20 μ l of Lysozyme (20 mg/ml, not provided) to resuspend the pellet if gram-positive bacteria or unknown strains are present, and incubate at 37°C for 30 min before moving to the next step.
 - Add 175 μ l of **Resuspension Buffer**, 20 μ l of Lysozyme (20 mg/ml, not provided) and 5 μ l of Lysostaphin (2 mg/ml, not provided) to resuspend the pellet if lysozyme-resistant gram-positive bacteria are present, and incubate at 37°C for 30 min before moving to the next step.
2. Add 100 μ l of the **PS Beads** and gently mix by inverting the tube several times. Incubate at 56°C for 5 min. **Note:** Resuspend PS Beads completely before use.
 3. Add 100 μ l of the **KG Buffer**, mix by gently rotating/inverting the tube until the beads clump is formed, usually take 1-3 min.
 4. Put samples on a magnet and incubate for 4 min. Carefully transfer supernatant to new 1.5 ml tubes.
 5. Add 220 μ l of the **BC Beads** and mix by vortexing. Incubate at 56°C for 5 min. **Note:** Resuspend BC beads completely before use.
 6. Put samples on the magnet and remove the supernatant.
 7. Add 800 μ l of **80% ethanol**. Incubate for 2 min. Discard supernatant completely. Repeat Step 7 one more time.
 8. Remove any residue solution using a pipette with fine tips. Dry samples for 5 min.
 9. Resuspend the sample in 20-50 μ l **Nuclease-free Water**. Put the samples on the magnet and transfer the supernatant (containing RNA) to new tubes.

Troubleshooting

Low RNA concentration

1. It is best to use bacterial cultures just before they reach maximum density. Do not undergrow or overgrow the cultures.
2. Resuspend completely after adding the Resuspension Buffer.
3. Resuspend PS Beads and BC beads completely before use.
4. Mix completely after adding the BC Beads. The BC Beads is viscous and make sure it is completely mixed with samples.
5. Make sure all beads are resuspended in water during the elution step.

DNA contamination

1. Mix gently after adding the PS Beads by inverting the tube several times. DO NOT shake the tubes.
2. Mix gently after adding the KG Buffer by rotating/inverting the tube until the beads clump is formed. DO NOT shake the tubes.
3. Carefully transfer the supernatant, but not the pellet, to new 1.5 ml tubes in step 4.

Low O.D. 260/230

1. Discard supernatant completely when washing (steps 6 and 7).
2. Remove any residue solution using a pipette with fine tips at step 8.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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