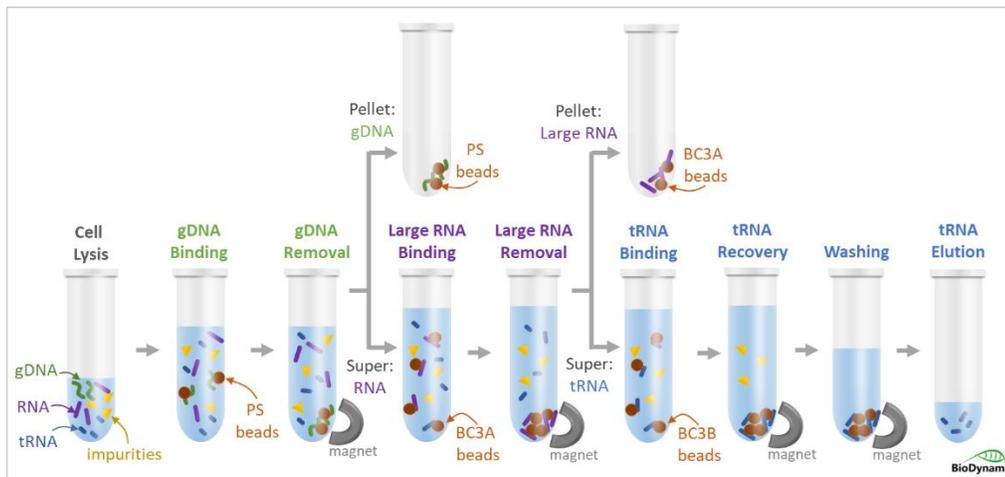


Bacterial tRNA Extraction Kit (Magnetic Beads)

Catalog No.	50342S	50342L
Preps	10 preps	50 preps

Description

The **Bacterial tRNA Extraction Kit (Magnetic Beads)** enables efficient microbial tRNA extraction from gram-negative and gram-positive bacteria, fungi, and yeast using advanced magnetic bead technology. The procedure is fast and reliable, yielding high-quality tRNA free from contamination by larger RNA species such as mRNA, 16S rRNA, and 23S rRNA.

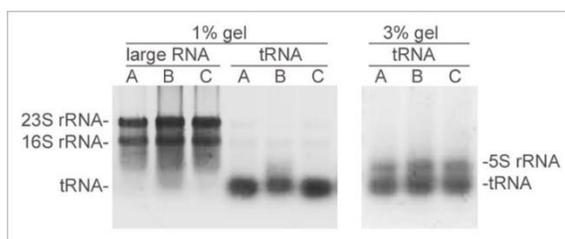


The kit employs a sequential purification process: genomic DNA is removed first, followed by the elimination of large RNA (mRNA, 16S rRNA, and 23S rRNA), after which tRNA is purified and eluted.

Microorganism samples are initially lysed using a lysis buffer combined with the first beads. Genomic DNA binds to these beads and is discarded, while the supernatant containing both large RNA and tRNA proceeds to the next step. This supernatant is then mixed with the second beads, which selectively bind to large RNA but not tRNA. The large RNA bound to these beads is discarded, leaving a supernatant enriched with tRNA. Finally, this supernatant is mixed with the third beads, which bind and purify the tRNA before elution.

The kit is free of toxic substances such as phenol and chloroform and allows for simultaneous processing of multiple samples.

Purified tRNA is free of genomic DNA, large RNA (mRNA, 16S rRNA, and 23S rRNA), and other contaminants including salts, lipids, and proteins. tRNA extracted using this kit is suitable for various downstream applications, including next-generation sequencing (NGS), hybridization, qPCR, and RT-PCR.



Three RNA samples. Left panel: both the large RNA fraction and tRNA fraction are shown on a 1% agarose gel to demonstrate the separation of the tRNA fraction from the large RNA fraction. Right panel: the tRNA fraction is shown on a 3% agarose gel. A small amount of 5S rRNA is present in the samples due to its small size, similar to tRNA.

Specifications	
Technology	Magnetic beads
Starting volume	0.4 – 2 ml (up to 10 ⁹ bacteria cells)
Starting material	Gram-positive; gram-negative; yeast; fungi
Elution volume	10-50 µl
O.D. 260/280	Around 2.0
O.D. 260/230	1.6-2.0

Features

- High-quality tRNA extraction
 - Without large RNA (mRNA, 16S rRNA, 23S rRNA etc.)
 - 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.	50342S	50342L
Resuspension Buffer (containing RNase inhibitor)	2 ml	10 ml
PS Beads	0.55 ml	2.75 ml
KG Buffer	0.3 ml	1.5 ml
BC3A Beads	0.8 ml	4 ml
BC3B Beads	3.8 ml	19 ml
Nuclease-free Water	0.5 ml	2.5 ml

Storage Condition

- Store Resuspension Buffer, BC3A Beads, and BC3B 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 and 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) 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) if lysozyme-resistant gram-positive bacteria are present, and incubate at 37°C for 30 min before moving to the next step.

2. Add 55 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 30 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 190 μ l supernatant to new 1.5 ml tubes.
5. Add 80 μ l of the **BC3A Beads** and mix by vortexing. Incubate for 5 min. **Note:** Resuspend BC3A beads completely before use.
6. Put samples on the magnet and transfer the supernatant to new 1.5 ml tubes. Discard beads pellet.
7. Add 380 μ l of the **BC3B Beads** and mix by vortexing. Incubate for 10 min. **Note:** Resuspend BC3B beads completely before use.
8. Put samples on the magnet, incubate for 5 min, and remove the supernatant carefully without touching the beads.
9. Add 800 μ l of **80% ethanol**. Incubate for 2 min. Discard supernatant completely. Repeat Step 9 one more time.
10. Remove any residue solution using a pipette with fine tips. Dry samples for 5 min.
11. Resuspend the sample in 10-50 μ l **Nuclease-free Water**. Put the samples on the magnet and transfer the supernatant (containing RNA) to new tubes.

Troubleshooting

Low tRNA 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 BC3B Beads completely before use.
4. Mix completely after adding the BC3B Beads. The BC3B 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.

Large RNA 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. Mix completely after adding the BC3A Beads. The BC3A Beads is viscous and make sure it is completely mixed with samples.
4. Carefully transfer the supernatant, but not the pellet, to new 1.5 ml tubes in steps 4 and 6.

Low O.D. 260/230

1. Discard supernatant completely when washing (steps 8 and 9).
2. Remove any residue solution using a pipette with fine tips at step 10.

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 biodynamy.com for Material Safety Data Sheet of the product.

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