

Column-Pure Fungi RNA Isolation Kit

Catalog
TBS6100

Unit
50 Preparations

Description

Column-Pure Fungi RNA Isolation Kit is designed for preparation of high quality total RNA from a wide range of fungal species. Fungal samples are lysed and homogenized by Buffer Rlysis-FG. RNA in the whole homogeneity is selectively absorbed on the spin column and other impurities are washed away. Total RNA is eluted from the membrane in the presence of RNase-Free Water in the final step.

3-5 µg total RNA can be purified from 30 mg filamentous fungi using this kit. Purified RNA is ready for most downstream applications such as RT-PCR, Northern Blotting, Poly A+ purification, nuclease protection and in vitro translation.

Component

| Product Component | Quantity (50 Preps) |
|--|---------------------|
| Buffer Rlysis-FG | 25 ml |
| Universal GT Solution* | 18 ml |
| Universal NT Solution* | 6 ml |
| RNase-free Water | 5 ml |
| Column-Pure Spin Column (with 2.0-ml Collection Tube) | 50 |

*Universal GT Solution and Universal NT Solution are supplied in a concentrated form. Before use, add 12 ml 96-100% ethanol to 18 ml concentrated Universal GT Solution and 24 ml 96-100% ethanol to 6 ml concentrated Universal NT Solution to make the complete solutions.

Store at 4°C

Features

- Fast. Using a rapid spin-column format, the entire procedure takes approx 20 minutes.
- High quality of RNA. OD_{260/280} of purified RNA is generally >1.8.
- Intact RNA: NO RNA degradation and integrity maintained.
- Economical. Simple, cost effective method of RNA isolation.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- RNase-Free pipettes and pipette tips
- Vortexer
- RNase-Free Ethanol (96-100%)
- RNase-Free Microcentrifuge tubes (1.5 ml or 2 ml)

Protocol

1. Add 350 µl Buffer Rlysis-FG into a RNase-Free centrifuge tube.
2. Grind cell pellets collected from 0.1~2.0 ml of fungal culture by centrifugation or 100-500 mg (wet weight) mycelia/spores in liquid nitrogen using a pestle. Transfer the ground sample to the RNase-Free centrifuge tube from step 1.
3. Incubate at room temperature for 5 minutes to make sure the cells are completely lysed.
4. Add a 1/2 volume of ethanol, mix by inverting the tube.
5. Transfer the solution to the spin column that is placed into a Collection Tube. Centrifuge at 12,000 × g for 30 seconds at room temperature, discard the flow-through in the collection tube.
6. Add 0.5 ml of Universal GT Solution to the column, centrifuge at 12,000 × g for 30 seconds at room temperature, discard the flow-through in the collection tube.
7. Add 0.5 ml of Universal NT Solution to the column, centrifuge at 12,000 × g for 30 seconds at room temperature, discard the flow-through in the collection tube.
8. Centrifuge the column at 12,000 × g for additional 30 seconds at room temperature.
Note: This step is very important to remove the residual ethanol.
9. Place the column in a new RNase-Free centrifuge tube. Add 50 µl RNase-Free Water. Keep at room temperature for 2 minutes. Centrifuge at 12,000 × g for 30 seconds at room temperature, store the collected RNA at -80°C

Note

Care must be taken when working with RNA. It is important to maintain an RNase-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNase-free tubes, tips, gels. Wear gloves at all times.

For research use only