

Exgene[™] Rice SV mini

Kit Contents

Contents	Size	Storage temperature
Buffer GL *	50 ml	Room temperature
Buffer PP	25 ml	Room temperature
Buffer TB *	35 ml	Room temperature
Buffer CW ** †	25 ml	Room temperature
Buffer AE ***	15 ml	Room temperature
Proteinase K ****	24 mg	-20°C
PK storage buffer	1.5 ml	Room temperature
EzSep [™] Filter column (Blue)	100 ea	Room temperature
Column Type G (Green)	100 ea	Room temperature
1.5 ml microcentrifuge tube	100 ea	Room temperature

* A precipitate can be formed in Buffer GL and TB under cold ambient condition. Heat the bottle at 37°C to dissolve completely in such a case.

** Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer CW as indicated on the bottle.

† Contains sodium azide as a preservative.

*** 10 mM TrisCl, pH 9.0, 0.5 mM EDTA.

**** After reconstitution of Proteinase K with 1.5 ml PK storage buffer, store at 4°C or -20°C.

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Protocol

Before experiments

- Prepare water or dry bath at 56°C.
- A precipitate can be formed in Buffer GL or TB under cold ambient condition. Heat the bottle at 37°C to dissolve completely in such a case.
- Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer CW as indicated on the bottle.
- After reconstitution of Proteinase K with 1.5 ml PK storage buffer, store at 4°C or -20°C.

1. Put a piece of grain of rice and one metal bead (5-6 mm) into a 2 ml screw tube. Pulverize a grain using appropriate bead-beater or milling machine.
2. Add 400 µl of Buffer GL and 10 µl of proteinase K into the tube, mix by vortexing.
3. Incubating at 56°C for 30 min.
4. Cool down the tube, apply 140 µl of Buffer PP, and vortex vigorously for 15 sec.
5. Transfer all lysate to a EzSep[™] Filter column.
6. Centrifuge at 14,000 x g for 2 min and transfer 400 µl of passed-through solution into a new 1.5 ml tube.
7. Apply 250 µl of Buffer TB and mix completely by vortexing.
8. Transfer the mixture to a mini column, centrifuge at 14,000 x g for 30 sec, empty the collecting tube and re-insert the mini column back to the tube.
9. Apply 650 µl of Buffer CW to the mini column, centrifuge at 14,000 x g for 30 sec, empty the collecting tube and re-insert the mini column back to the tube.
10. Apply 300 µl of Buffer CW to the mini column, centrifuge at 14,000 x g for 1 min and transfer the mini column into a new 1.5 ml tube.
11. Apply 50 µl of Buffer AE to the center of mini column membrane. Let it stand for 1 min and centrifuge at 14,000 x g for 1 min.