SunLong Biotech Co.,LTD

Tel: 0086-571-56623320 Fax:0086-571-56623318

E-mail: sales@sunlongbiotech.com

www.sunlongbiotech.com

Universal Genomic DNA Extraction Kit

Cat No.: EXD0207

Package: 50T/100T

Storage: At room temperature(15℃-25℃) in dry place for 1 year.

Kit Contents:

Component	EXD0207-50T	EXD0207-100T
RNase A	1 ml	1 ml × 2
Proteinase K	1 ml	1 ml × 2
Solution A	25 ml	50 ml
Solution B	25 ml	50 ml
Washing buffer	15 ml	15 ml × 2
Elution buffer	15 ml	30 ml
Adsorption column	50 Units	100 Units
Collection tube	50 Units	100 Units
Instruction	1 Piece	1 Piece

Note: RNase A and Proteinase K should be kept at -20℃.

Product Description

Universal Genomic DNA Extraction Kit is suitable for extracting of genomic DNA form soil, stool, insect and other samples. This kit has a good lysis effect on soil, stool, insect and other samples to preserve biological DNA diversity to the utmost.

The extracted DNA is large yield and good integrity, it can be directly used for a variety of routine operations, including enzyme digestion, PCR, library construction, Southern blot, etc.

Protocol

Add fresh opened absolute ethanol in Washing buffer before use, volume is based on the label of bottle as a reference. Put cap back on bottle and shake well. All centrifuge steps are performed at room temperature(15℃-25℃).

1. Treated samples:

1) Soil: weigh 0.1-0.3g(based on dry-wet change) soil and add soil into mortar, pour proper amount of liquid nitrogen, grind immediately, repeat three times. When soil turns into powder, add 500ul Solution A, keep shaking until solute is completely suspended.

2) Stool: weigh 0.1-0.3g(based on dry-wet change) stool, add 500ul Solution A, keep shaking until solute is completely suspended.

3) Insect: weigh 0.1-0.3g insect, proper amount of liquid nitrogen, grind immediately, repeat three times. When insect turns into powder, add 500ul Solution A, keep shaking until solute is completely suspended.

4) Unknown sample: If it is powder, weigh 0.1-0.3g(based on dry-wet change) directly, add 500ul Solution A. If it is piece, weigh 0.1-0.3g sample and add soil into mortar, pour proper amount of liquid nitrogen, grind immediately, repeat three times. When soil turns into powder, add 500ul Solution A, keep shaking until solute is completely suspended.

2. Add 20ul RNase A(10mg/ml) to suspension, place at 55℃ for 10min .

3. Add 20ul Proteinase K(10mg/ml), mix thoroughly, digest at 55℃ water bath for 30min. During digestion, invert centrifuge tube several times to mix, centrifuge at 12,000rpm for 10min. Transfer supernatant to a new centrifuge tube. If there is precipitation, centrifuge again.

4. Add 500ul Solution B, mix well. If white precipitation exists, place at 55℃ for 5min, precipitation will disappear, it doesn't affect the follow-up operations. If solution is not clear, indicating that sample digestion is not complete, it may lead to low and impure extracted DNA, it may also lead to blocking Adsorption column, please increase digestion time.

5. Add 500ul absolute ethanol, mix well, it may appear flocculation precipitation, it does not affect DNA extraction. Add liquid in Adsorption column, place for 2min (add in twice, each 700ul).

6. Centrifuge at 12,000rpm for 2min, abandon waste liquid, put Adsorption column into Collection tube.

7. Add 600ul Washing buffer to Adsorption column(don't forget to add absolute ethanol), centrifuge at 12,000rpm for 1min, abandon waste liquid, put Adsorption column into Collection tube.

8. Add 600ul Washing buffer to Adsorption column, centrifuge at 12,000rpm for 1min, abandon waste liquid, put Adsorption column into Collection tube.

9. Centrifuge at 12,000rpm for 2min, place Adsorption column at room temperature(15℃-25℃) or 50℃ warm-box for a few minutes to remove residual Washing buffer in Adsorption column, otherwise ethanol in Washing buffer will affect the follow-up experiments such as enzyme digestion and PCR.

10. Put Adsorption column into a clean centrifuge tube, drop 50-200ul Elution buffer preheated at 65℃ water bath in center of adsorption membrane(tip don't touch membrane), place at room temperature(15℃-25℃) for 5min, centrifuge at 12,000rpm for 2min.

11. Add Elution buffer got from step 10 centrifuge tube to Adsorption column, place at room temperature(15℃-25℃) for 2min, centrifuge at 12,000rpm for 2min, obtain high quality genomic DNA .

Notes

1. Because of different samples, final extracted DNA content and purity are different. In a general way, if extracted DNA can't be detected by electrophoresis, the reason is that extracted DNA concentration is low, but it can be used for PCR and get a good result. Please use fresh sample, otherwise, extracted DNA fragment will be small and less.

2. If component of kit appears precipitation, redissolve at 65℃ water bath, precipitation will disappear, it does not affect DNA extraction.

3. If sample digestion is not complete, Adsorption column might be blocked during centrifuge, properly prolong centrifuge time.

4. Volume of Elution buffer shouldn't be less than 50ul, if volume is too small, it will affect recovery efficiency. pH value of the Elution buffer may also affect elution efficiency, if using water as Elution buffer, please ensure pH8.0(it can use NaOH to adjust pH value), elution efficiency will be reduced if pH value is lower than 7.0. DNA product should be stored at -20℃ to prevent DNA degradation.

5. DNA concentration and purity detection(higher concentration): size of obtained genomic DNA fragment is related with storage time, shear force and other factors. The concentration and purity of obtained DNA fragment can be detected by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak at OD260. When OD260= 1, it is equal to 50μg/ml of double-stranded DNA and 40μg/ml single-stranded DNA. OD260 / OD280 ratio should be 1.7 to 1.9, if deionized water is used instead of Elution buffer during elution operation, ratio will be lower, because pH value and ion will affect absorbance, but it does not mean low purity.