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Marine animal Tussie DNA Kit

Product Number: DNK3701

Shipping and Storage

1. Buffer CB or Buffer SL may precipitate and precipitate at low temperatures. It can be dissolved again in a water bath at 37℃ for a few minutes to restore clarity and transparency. After cooling to room temperature, it can be used.
2. Proteinase K is stored in a ready to use glycerol buffer and transported at room temperature. After receipt, store at room temperature not exceeding 25℃ for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DNK3701 50 Preps	DNK3701 100 Preps	DNK3701 200 Preps
Balance buffer	RT	5 ml	10 ml	20 ml
Buffer SL	RT	11 ml	20 ml	40 ml
Buffer CB	RT	11 ml	20 ml	40 ml
Buffer PE	RT	16 ml	32 ml	64 ml
		Add absolute ethanol as instructed on the bottle label before first use		
Buffer WB	RT	13 ml	25 ml	50 ml
		Add absolute ethanol as instructed on the bottle label before first use		
Buffer EB	RT	15 ml	15 ml	20 ml
Proteinase K	4℃	1 ml	1ml×2	1ml×4
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Except for low-temperature components, this kit can be stored at room temperature for 12 months without affecting performance.

Description

The proprietary binding buffer combined with Proteinase K rapidly lyses cells. DNA is specifically adsorbed by silica membrane spin columns. No toxic reagents such as phenol or chloroform are required, nor time-consuming alcohol precipitation steps. Proteins and other inhibitory impurities are maximally removed. This kit enables efficient extraction of genomic DNA from tissues of various marine animals, fish and shellfish. The extracted DNA can be directly applied to restriction enzyme digestion, PCR, Southern Blot, virus detection and other experiments.

Application

Suitable for rapid extraction of genomic DNA from tissues of various marine animals, fish, shellfish and other organisms.

Features

1. No toxic reagents like phenol required; no time-consuming ethanol precipitation steps.
2. Fast and simple workflow; processing of a single sample can be completed within 30 minutes generally.
3. Multiple column washes guarantee high DNA purity, with typical OD260/OD280 ratio ranging from 1.7 to 1.9. Recovered DNA fragment length reaches 30 kb–50 kb, which can be directly used for PCR, Southern blot and various restriction digestion reactions.

Note

1. All centrifugation steps are performed at room temperature using a bench-top centrifuge with a maximum speed of 13,000 rpm.
2. Prepare isopropanol in advance (isopropanol is the preferred reagent for the corresponding step; ethanol may be used as a substitute if isopropanol is unavailable). RNase A is optional.
3. Preheat the required water bath to 55°C before starting the experiment.

Protocol(Please read the precautions before the experiment)

Tip: Before the first use, please add the specified amount of anhydrous ethanol to Buffer WB and Buffer PE and mix well. After adding, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid adding it multiple times!

1. Cut no more than 30mg of tissue material and place it in a 1.5ml centrifuge tube containing 180µl tissue buffer SL, vortex oscillation for 15 seconds.

   According to the different extracted tissues, the starting amount may also vary slightly. The amount of cells in the gills is relatively large, and it is generally recommended to extract no more than 20mg. If cracking is difficult, it can be ground with liquid nitrogen first.
2. Add 20µl of Proteinase K (20mg/ml) and immediately vortex oscillate to mix thoroughly. Place the lysate in a 55℃ water bath for 1-3 hours or until the tissue is completely digested.

   Different tissues require different lysis times, usually taking 0.5-2 hours to complete. Scallop tissues can be completely cleaved within 0.5 hours, while shrimp and fish tissues can be cleaved within 1 hour. Shake the mixed sample 2-3 times per hour, and mix well for 15 seconds each time.

   Optional steps: If there is a lot of RNA residue and RNA needs to be removed, you can add 20µl of RNase A (25mg/ml) solution after completing step 2, shake well, and let it sit at room temperature for 5-10 minutes.
3. Add 200µl Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70℃ for 10 minutes.
4. Column equilibration: Add 100 µL Equilibration Buffer to Adsorption Column AC, centrifuge at 13,000 rpm for 1 min, discard flow-through and set column aside for later use.

   Note: Equilibration buffer enhances nucleic acid binding capacity of silica membrane; use freshly equilibrated columns on the day of experiment.
5. After cooling, add 100µl of isopropanol, thoroughly invert or vortex shake and mix well. At this time, flocculent precipitation may occur.

   Note: Immediate and thorough mixing by vortex or pipetting is critical. Insufficient mixing will drastically reduce DNA yield. If the sample is viscous and hard to mix, vortex for 15 sec to homogenize fully.
6. Add the previous mixture and possible precipitates to an adsorption column AC, centrifuge at 13000rpm for 60 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube.

   If there is insoluble tissue that may block the gun head, the gun head can be gently rubbed on absorbent paper to remove the insoluble material; If there is a small amount of mixture sucked up, the gun tip and insoluble substances can be discarded together. This method is to remove insoluble substances to avoid clogging the centrifuge column.
7. Add 500µl Buffer PE (verify ethanol has been added in advance!) to the column, centrifuge at 13,000 rpm for 30 sec, discard waste liquid.
8. Add 600µl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
9. Repeat Step 8 once.
10. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
11. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100µl Buffer EB to the middle of the adsorption membrane (Buffer EB is better preheated in a 65-70℃ water bath), leave at room temperature for 3-5 minutes, and centrifuge at 12000rpm for 1 minute. Add the obtained solution back into the centrifugal adsorption column, let it stand at room

temperature for 2 minutes, and centrifuge at 12000rpm for 1 minute.

Re-load the eluate from the first centrifugation back onto the column, incubate at room temperature for 2 min, and centrifuge at 13,000 rpm for 1 min to increase DNA concentration by approximately 10%.

The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50µl. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.

12. DNA can be stored at 2-8 ℃, and if it needs to be stored for a long time, it can be placed at -70℃.