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Marine animal Tussie DNA Kit

Product Number: DNK3701

Shipping and Storage

1. Buffer CB or Buffer IR 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
Buffer SL	RT	11 ml	20 ml	40 ml
Buffer CB	RT	11 ml	20 ml	40 ml
Buffer IR	RT	25 ml	50 ml	100 ml
Buffer WB	RT	13 ml	25 ml	50 ml
Buffer EB	RT	15 ml	15 ml	15ml×2
Proteinase K	4℃	1 ml	1ml×2	1ml×4
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

The unique Buffer CB/Proteinase K rapidly cleaves cells and inactivates intracellular nucleases, and then selectively adsorbs genomic DNA onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer IR and Buffer WB remove impurities such as cell metabolites and proteins. Finally, low salt Buffer EB elutes pure genomic DNA from the silica matrix membrane.

Application

Suitable for rapid extraction of genomic DNA from various marine animal tissues

Note

Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH can affect elution efficiency. Wash DNA with water and store it at -20℃. If DNA needs to be stored for a long time, it can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0). However, EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

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 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. After cooling, add 100μl of isopropanol, thoroughly invert or vortex shake and mix well. At this time, flocculent precipitation may occur.
5. 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.

   It is very important to immediately vortex or blow thoroughly in the above steps. Insufficient mixing seriously reduces production. If necessary, if the sample is viscous and difficult to mix, vortex oscillation can be used for 15 seconds to mix.
6. Add 500μl Buffer IR, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
7. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
8. Add 600μl Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
9. 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.
10. 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.

    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.
11. DNA can be stored at 2-8 ℃, and if it needs to be stored for a long time, it can be placed at -20℃.