MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Oral/Pharynx Swab Fast DNA Kit

Product Number: DNK3001

Shipping and Storage

1. Buffer CB or Buffer IR may precipitate and precipitate at low temperatures, and can be dissolved again in a water bath at 37 ℃ for a few minutes. After restoring clarity and transparency, it can be cooled to room temperature before use.
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 after use.

Components

Component	Storage	DNK3001 50 Preps
Balance Buffer	RT	5 ml
Buffer ML	RT	20 ml
Buffer CB	RT	20 ml
Buffer IR	RT	25 ml
Buffer WB	RT	13 ml
Poly Carrier	–20°C	200μl
Buffer EB	RT	15 ml
Proteinase K(20mg/ml)	4°C	1ml
Adsorption column AC and collection pipe	RT	50

Note:Buffer WB-Add the specified amount of ethanol according to the instructions before the first use.

Description

This kit uses a specially designed imported DNA adsorption column and a unique buffer system, making it particularly suitable for separating and purifying genomic DNA from oral/pharyngeal swabs.After lysis and digestion of samples from various sources, DNA is selectively adsorbed onto the silica matrix membrane in a highly dissociated salt state (especially equipped with Poly Carrier to easily capture trace amounts of nucleic acids from the system). Then, impurities such as salt, cell metabolites, proteins, etc. are removed through a series of rapid rinsing centrifugation steps. Finally, low salt elution buffer is used to elute pure genomic DNA from the silica matrix membrane.The purified DNA is free of impurities and PCR inhibitors, and can be directly used for PCR analysis. Typical yield 0.5μg-3.5μg/swab.

Features

1. There is no need to use toxic reagents such as phenol, nor do steps such as ethanol precipitation.
2. Saves time, is simple, and a single sample operation can generally be completed within 20 minutes.
3. Equipped with a Poly Carrier for fully collecting special trace amounts of DNA.
4. Multiple column rinsing ensures high purity, and the extracted DNA has high purity and stable and reliable quality, making it suitable for various routine operations, including PCR, enzyme digestion, sequencing, Southern hybridization, etc.

Application

Suitable for isolating and purifying genomic DNA from oral/pharyngeal swabs.

Note

For Research Use Only

1. **All centrifugation steps are completed at room temperature** using a traditional desktop centrifuge with a rotational speed of up to 13000rpm.
2. Before starting the experiment, preheat the required water bath to a specific temperature for later use.
3. Buffer CB and Buffer IR contain irritating compounds. Wear latex gloves during operation to avoid contact with skin, eyes, and clothing. If it comes into contact with the skin or eyes, rinse with plenty of water or physiological saline.
4. Poly Carrier:

How to use Poly Carrier:If the initial processing volume is small (there are very few cells collected from oral pharyngeal swabs), we recommend using Poly Carrier. If a large amount of DNA production is expected, users can choose whether to add PolyCarrier according to their needs.When using, add 4μl Poly Carrier into 400μl Buffer CB required for each sample extraction, and mix the Buffer CB and Poly Carrier solution completely upside down (Buffer CB is easy to form foam, do not use vortex oscillation to mix).

Use of balance buffer

1. Description

During the long-term placement of nucleic acid adsorption silica gel membrane columns, they react with charges/dust in the air and affect their nucleic acid binding ability.After pre-treatment with equilibrium solution, the silica gel column can greatly reduce the hydrophobic groups of the silica gel membrane in the column and improve the binding ability of nucleic acids.Thus improving the recovery efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution. If accidentally touched, please clean it with a large amount of tap water.After use, the bottle cap needs to be tightly closed to avoid contact with air. Store at room temperature. During storage, there may be precipitation formation. Please heat to 37 ℃ to completely eliminate the precipitation.

2. Protocol

Take a new silica gel membrane adsorption column and install it in a collection tube, and suck 100μl of equilibrium solution into the column.Centrifuge at 13000rpm for 1 minute, discard the waste liquid from the collection tube, and reposition the adsorption column in the collection tube. At this point, the balance liquid pre-treatment column is completed. Follow the subsequent operating steps.

Protocol(Please read the precautions before the experiment)

Note:Before the first use, please add the specified amount of anhydrous ethanol to the rinsing solution WB and mix thoroughly. After addition, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid multiple additions!

1. Sample collection

1.1. Processing method for oral and pharyngeal swab samples: Take a medical disinfectant cotton swab (do not touch the degreased cotton area with your hands), extend it into the oral cavity, and scrape it back and forth 20 times against the inner side of the cheek (rotate the cotton swab occasionally), fully touching the oral mucosa. Use scissors to cut off the cotton swab from its stem, place it in a 2 mL centrifuge tube, and add 400μl Buffer ML.

Note:Before collection, rinse your mouth gently with water. To prevent contamination of the sample with food or beverages, eating or drinking should be avoided within 30 minutes before sampling.

1.2. Processing method for nasopharyngeal swab samples: Cut and transfer the swab that has been wiped in the throat to a 2 ml centrifuge tube, and add 400μl of Buffer ML.

1.3. Saliva sample processing method: Take saliva as required and transfer it to a 5 ml centrifuge tube, add an equal volume of Buffer ML, and mix well through vortex oscillation. Take 400μl of sample for subsequent extraction.

1.4. Swab treatment method stored in swab preservation solution: Add 1/5 volume of lysis solution ML to the swab preservation solution. Take 400μl of sample for subsequent extraction.

2. Add another 20μl of Proteinase K (20mg/ml) solution, immediately vortex shake and thoroughly mix.

Optional steps (generally not required): Leave at 56℃ for 30 minutes, and vortex mix for 10 seconds every 10 minutes. **In general, this step can be omitted, unless the effect is not good, try adding this step again.**

3. Add 400μl Buffer CB, immediately swirl and **mix thoroughly**, and let stand at 70 ℃ for 10 minutes. At this point, the solution

strain becomes clear.

If the number of cells on the swab is low, resulting in an extracted genomic DNA yield of less than 1μg, 4μl Poly Carrier can be added to 400μl Buffer CB.

Balance Buffer pre-treatment adsorption column backup:The use of a Balance Buffer for pre-treatment of silica gel membrane adsorption columns is a necessary step, and the specific method can be found in the previous section "About the Use of Balance Buffer"

4. After cooling, add 200μl of anhydrous ethanol and **immediately vortex oscillate to thoroughly mix**. Short centrifugation to remove droplets from the inner wall of the tube cover and collect all liquids to the bottom of the tube.

If the surrounding environment is above 25 ℃, ethanol needs to be pre cooled on ice before being added.

5. Add the mixture from the previous step to an adsorption column AC, centrifuge at 12, 000rpm for 30s, and discard the waste liquid from the collection tube.
6. Add 500μl Buffer IR, centrifuge at 12, 000rpm for 30 seconds, and discard the waste liquid.
7. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12, 000rpm for 30 seconds, and discard the waste liquid.
8. Add 600μl Buffer WB, centrifuge at 12, 000rpm for 30 seconds, and discard the waste liquid.
9. Return the adsorption column AC to the empty collection tube, centrifuge at 12, 000rpm for 2 minutes, and try to remove the rinsing solution as much as possible to prevent residual ethanol from inhibiting downstream reactions in the rinsing solution.
10. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 20-50μl Buffer EB to the middle of the adsorption membrane, and let it sit at room temperature for 2 minutes. Centrifuge at 12, 000rpm for 1 minute. Collect the filtrate, which is the DNA solution.

Washing and injection

The recovery yield can be increased by:

1) preheating the eluent at 70℃;

2) Place the DNA filtrate on the column again, let it sit at room temperature for 2 minutes, and then elute.

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

Problems and Solutions

Problem	Comments and Suggestions
Low DNA production or absence of DNA in the eluent	1. Poly Carrier has not been added to Buffer CB. Suggestion: Carefully read Note 4. 2. Sample freeze-thaw more than once. Suggestion: Try to use fresh samples and samples with freeze-thaw no more than once. 3. The sample has been left at room temperature for too long Suggestion: it is recommended to process the sample as soon as possible or store it in a suitable low-temperature manner. 4. Incomplete lysis, Proteinase K has failed. Suggestion: After receiving Proteinase K, pack and freeze according to the amount used each time to avoid repeated freeze-thaw. 5. Buffer CB and Poly Carrier were not thoroughly mixed. suggestion: thoroughly vortex mixed. 6. Reagents and samples not thoroughly mixed. Suggestion: After adding each reagent, thoroughly mix. 7. Low elution efficiency. Suggestion: Ensure that step 10 is done, otherwise residual ethanol will affect elution efficiency. Carefully read step 11 and only use Buffer EB for elution.

Downstream reactions of DNA, such as PCR, are ineffective	1. Low DNA production or absence of DNA in the eluent. Suggestion: increase the amount of DNA used in downstream reactions. 2. Reduced sensitivity Suggestion: Determine the maximum allowable amount of Buffer EB in downstream PCR applications, reduce or increase the amount of Buffer EB used in PCR reactions, and adjust the volume of DNA elution accordingly.
DNA downstream enzyme cleavage cannot be cut or incomplete	1. The residual ethanol in the centrifuge column or the accidental presence of ethanol at the bottom inhibited the enzyme digestion reaction. Suggestion: Make sure to follow step 10 and let the remaining ethanol evaporate by letting it air for a few minutes. 2. Some silicon-based plasma membrane components were eluted together, inhibiting the enzymatic cleavage reaction. Suggestion: Centrifuge the washed genomic DNA solution at 12, 000rpm for another minute, and carefully remove the supernatant for use.