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

Blood Genomic MiniS Kit

Product Number: DNK0101

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. Upon receipt, it should be stored at room temperature for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years
3. 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	DNK0101 50 Preps	DNK0102 100 Preps	DNK0103 200 Preps
Balance Buffer	RT	5ml	10ml	20ml
Buffer CB	RT	15ml	30ml	60ml
Buffer IR	RT	25ml	50ml	100ml
Buffer WB	RT	13ml	25ml	50ml
Buffer EB	RT	15ml	15ml	20ml
Proteinase K	RT	1ml	1ml×2	1ml×4
Adsorption column AC	4°C	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

The unique Buffer CB and Proteinase K rapidly lyse cells and inactivate intracellular nucleases, and then selectively adsorb 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.

Features

1. It does not require the use of toxic reagents such as phenol, nor does it require steps such as ethanol precipitation.
2. Fast and simple, the operation of a single sample can generally be completed within 20 minutes.
3. Multiple column washes ensure high purity, with a typical yield of 200μl of whole blood, which can extract 3-12μg, and a typical OD260/OD280 ratio of 1.7-1.9, with a length of up to 30kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.
4. The red blood cell lysis fluid formula selected from over a dozen formulas is fast and complete, and customers can choose to purchase it according to their needs.
5. A typical yield of 200μl of whole blood can extract 3-12μg of genomic DNA.

Application

Suitable for rapid extraction of whole blood genomic DNA

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 silicone membrane adsorption column and place it in a collection tube. Take 100μl of Balance Buffer and transfer it into the column. Centrifuge at 13000rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube. At this point, the Balance Buffer has completed preprocessing the columns. Follow the subsequent operating steps.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000 rpm.
2. There may be significant differences in the number of white blood cells in different samples, especially in disease samples, so individual differences in yield may also be significant.
3. It is necessary to prepare isopropanol by oneself (for the steps of adding isopropanol, it is recommended to use isopropanol first, and ethanol can be used instead of isopropanol). 1× PBS (phosphate buffer, optional), RNase A (optional).
4. Preheat the required water bath to 70℃ for later use before starting the experiment.
5. For optimal results, it is best to use fresh blood specimens or specimens stored at 4℃ for less than 3 days. Do not use specimens that have been repeatedly freeze-thawed more than 3 times, otherwise it will seriously reduce production.
6. 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), but EDTA may undergo downstream enzyme digestion reactions and can be diluted appropriately when used.

Protocol(Please read the precautions before the experiment)

Note: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 multiple additions!

1. Take 200μl of fresh, frozen or mixed with various anticoagulants of blood and place it in a 1.5ml centrifuge tube.
   1.1. If the initial amount of whole blood is less than 200μl, supplement it with 1×PBS to 200μl. If the starting amount is between 200μl-300μl, subsequent operations need to increase the reagent dosage proportionally. If the starting amount is between 300μl-1 ml, red blood cell lysis operation needs to be carried out first (you can contact us to purchase red blood cell lysis solution).
   1.2. If the processed blood sample is anticoagulant blood from poultry, birds, amphibians, or lower level organisms, and the red blood cells are nucleated cells, the processing amount is only 5-20μl, and 1-PBS can be added to supplement 200μl before proceeding to the next steps
2. Add 20μl of Proteinase K (20mg/ml) solution, mix well, then add 200μl of Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70°C for 10 minutes. The solution strain is clear (but the color is slightly black).

   Optional steps, generally not required: If there is a lot of RNA residue and RNA needs to be removed, you can add 20μl of RNase A (10 mg/ml) solution before adding 200μl of Buffer CB, shake well, and let it sit at room temperature for 5-10 minutes.

   Balance buffer pre-treatment adsorption column backup:

   The pre-treatment of silica gel membrane adsorption column with equilibrium solution is a necessary step, and the specific method can be found in the previous section "Use of balance buffer"
3. After cooling, add 100μl of isopropanol (or anhydrous ethanol can be used as a substitute), immediately vortex and shake

thoroughly to mix well. At this time, flocculent precipitation may occur.

Note: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.

4. Add the previous mixture (including possible precipitates) to an adsorption column AC, centrifuge at 13000 rpm for 30-60 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube.
5. Add 500μl of Buffer IR, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
6. Add 600μl of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
7. Repeat step 6 once.
8. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
9. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100μl of Buffer EB to the middle of the adsorption membrane (it is better to preheat Buffer EB in a water bath at 80-100°C beforehand). Leave it at room temperature for 3-5 minutes and centrifuge at 13000 rpm for 1 minute.

   The solution obtained from the first elution can be re added to the centrifuge column, left at room temperature for 2 minutes, and centrifuged at 13000 rpm for 1 minute. It can increase the concentration by about 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 30μl. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.
10. DNA can be stored at -20°C, and if stored for a long time, it can be stored at -70°C