Micronbrane Medical

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DEVIN™ Microbial DNA Enrichment Kit

REF MEK-01-024

(For Research Use Only)

1. Intended Use

The Devin™ Microbial DNA Enrichment Kit is suitable for removing the white blood cells and isolating microbial DNA from whole blood, plasma or other body liquids. Extracted nucleic acids can be analyzed by downstream application, such as real-time PCR and/or next-generation sequencing.

2. Before starting the experiment

This kit is for **Research Use Only**.

- Read this manual carefully before starting the experiment. If something is not clear, please contact us first before starting the experiment.
- Double check if you have all the components ready before starting the experiment. If something is missing in the KIT, contact us immediately and don't proceed with the experiment.

3. Contents

One kit includes following:

1. Devin™ Fractionation Syringe Filter x 24 pcs
2. 96-well plate x 3 pcs (8x Rxns / pcs)
3. Elution buffer x 1 tube
4. Incubation buffer x 1 tube
5. Proteinase K x 1 tube
6. Lysozyme x 1 tube

4. Storage and shelf life

1. Except Lysozyme, all other components of the Kit can be stored under room temperature (15°C ~35°C) until the expiration date labeled on the box.
2. The Lysozyme is transported at room temperature. After received, please store at –20°C.

5. Required but not provided in the kit

- High speed centrifuge
- Tips (10µl, 200µl, 1000µl)
- Pipette (2µl, 10µl, 20µl, 100µl, 200µl, 1000µl)
- Syringe
- Falcon tube15 mL
- Falcon tube50 mL
- Vortex shaker
- Reagent storage
- Eppendorf 1.5ml
- Eppendorf magnetic rack 1.5ml
- Disposable gloves
- Scissors, utility knives
- Ethanol 95~100%
- Dry bath
- Refrigerator 4°C & –20°C

6. Precautions

1. Please wear mask and disposable gloves when handling reagents.
2. Use sterile consumables to avoid nuclease contamination.
3. Avoid eyes, skin and clothing contact with reagents. In case of any contact, flush with flowing water.
4. When the temperature is below 20°C, place the reagent plate in an oven (preheated 42°C~60°C) for 5 to 10 minutes.
5. Reagent solution contains guanidine salt, avoid using bleach and flush with water immediately after any contact.
6. Avoid vigorous shaking to avoid excessive foam formation.
7. Do not expose opened reagents or plates to air. The evaporation may change pH and thus affect the extraction effectiveness.
8. Reagents are all colorless and transparent. Colored reagents indicate contamination, please replace with a fresh plate before proceeding.

7. Sample preparation

Please follow the recommendations below for specimen preparation:

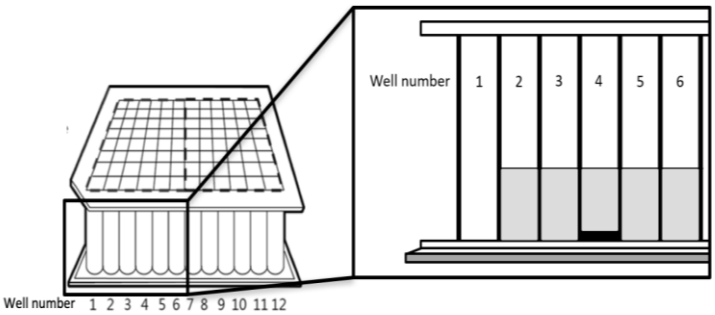
1. Prepare 3-5 mL of whole blood (or other body fluid) in EDTA
2. Maximum storage time for each specimen is 6 hours at ambient temperature.
3. If specimen is stored at 2~6 °C, storage time can be extended to 12 hours.

Note: DO NOT filter frozen blood sample to avoid filter blockage.

Sample pellet preparation:

1. Draw 3-5 mL of whole blood sample into the syringe.
2. Open the filter package and securely attach the filter to the syringe on the one side then assemble the needle on the other.
3. Filter the whole blood sample with Devin™ Fractionation Syringe Filter and collect into 15ml / 50ml centrifugation tube.
4. Centrifuge the whole blood sample at 400 x g for 15 minutes at room temperature. Collect the plasma (upper layer clear phase) and transfer to a new 15ml / 50ml centrifugation tube.
5. Centrifuge plasma at 16000 x g for 15 minutes at room temperature. Collect precipitation at the bottom of the centrifugation tube and use it as a **Sample pellet** in microbial DNA extraction described below.

8. Microbial DNA extraction (for manual use only)



Contents of 96-well plate:

Well No	Buffer Solution	Volume
2	Washing Buffer 1A (WB1A)	500 µl
3	Washing Buffer 1B (WB1B)	500 µl
4	Magnetic Beads (MB, with preservation buffer)	500 µl
5	Washing Buffer 2A (WB2A)	500 µl
6	Washing Buffer 2B (WB2B)	500 µl

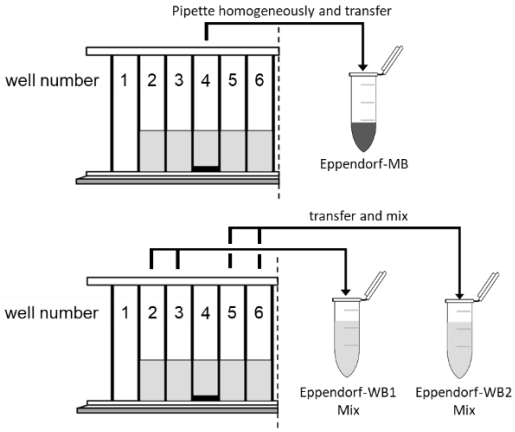
Step 1.

Remove aluminum foil carefully to avoid splashing and pipette well #4 buffer homogeneously.

Transfer well #4 buffer into a new Eppendorf and label MB on the cap.

Transfer well #2 and well #3 buffer into a new Eppendorf and label WB1 Mix on the cap.

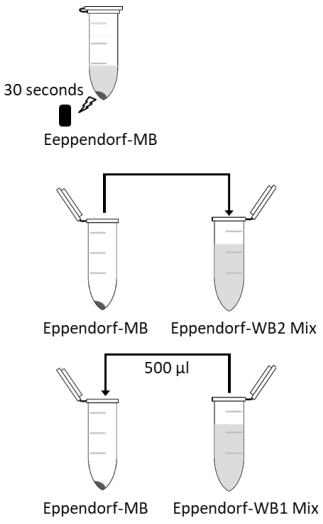
Transfer well #5 and well #6 buffer into a new Eppendorf and label WB2 Mix on the cap.



Step 2.

Put the Eppendorf MB on the magnetic rack for 30 seconds to absorb magnetic beads, and transfer supernatant to Eppendorf WB2 Mix.

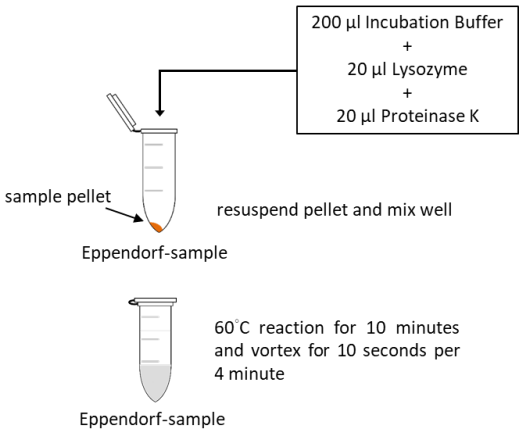
Remove the Eppendorf MB from the magnetic rack. Add 500 µl WB1 Mix and vortex 2 seconds, then spin down.



Step 3.

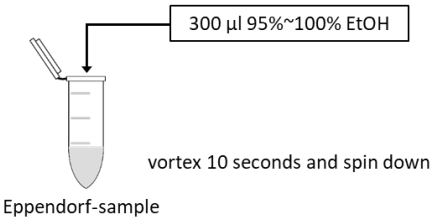
Add 20 µl Lysozyme + 20 µl Proteinase K + 200 µl Incubation Buffer into the sample pellet and resuspend it well.

React at 60°C for 10 minutes and vortex for 10 seconds per 4 minutes, then spin down.



Step 4.

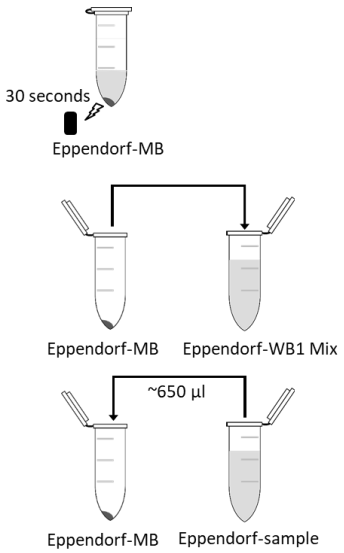
Add 300 µl 95%~100% EtOH. Vortex for 10 seconds and spin down.



Step 5.

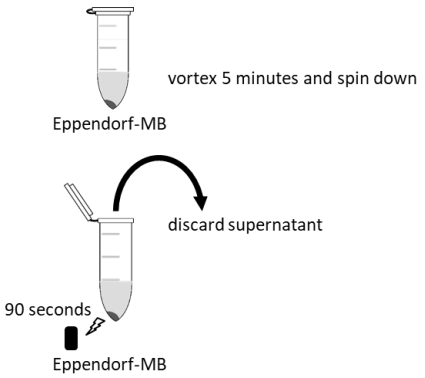
Put the Eppendorf MB on the magnetic rack for 30 seconds to absorb magnetic beads, and transfer supernatant to Eppendorf WB1 Mix.

Remove the Eppendorf MB from the magnetic rack and add the rest of buffer of Eppendorf Sample.



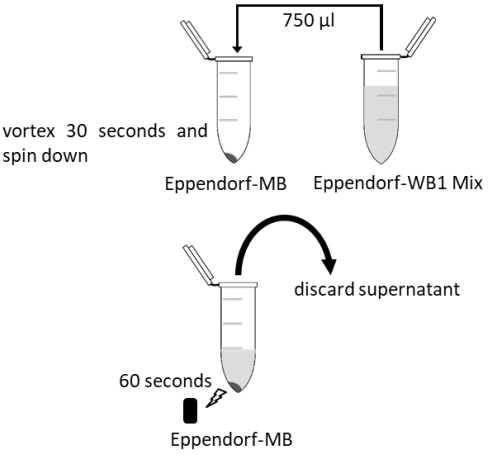
Step 6.

Vortex Eppendorf MB for 5 minutes and spin down. Put the Eppendorf MB on the magnetic rack for 90 seconds to absorb magnetic beads and discard supernatant.



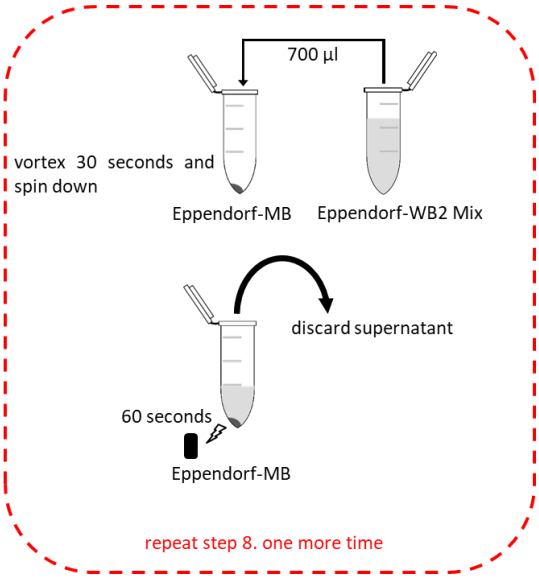
Step 7.

Add 750 µl WB1 Mix. Vortex for 30 seconds and spin down. Put the Eppendorf MB on the magnetic rack for 60 seconds to absorb magnetic beads and discard supernatant.



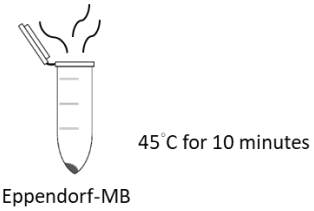
Step 8.

Add 700 µl WB2 Mix to Eppendorf-MB. Vortex for 30 seconds and spin down. Put the Eppendorf MB on the magnetic rack for 60 seconds to absorb magnetic beads and discard supernatant. Repeat step 8 one more time.



Step 9.

Open Eppendorf-MB, and heat at 45°C for 10 minutes to dry the magnetic beads.

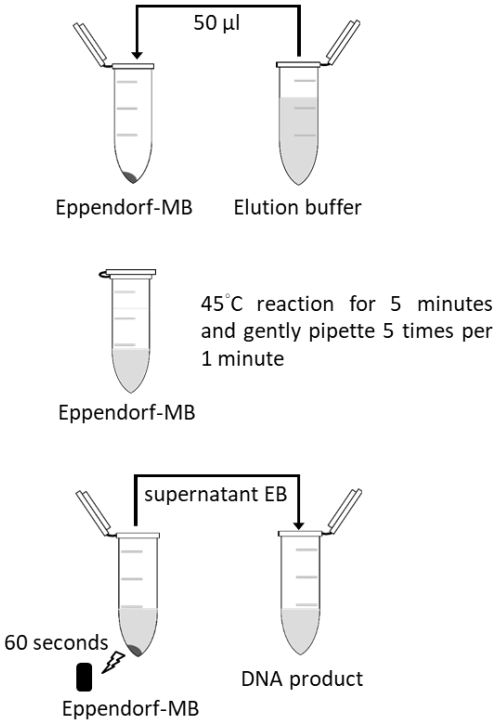


Step 10.

Add 50 µl of Elution buffer into Eppendorf MB and let them react at 45°C for 5 minutes, gently pipetting 5 times per minute.

After reaction completes, spin down and put the Eppendorf MB on the magnetic rack for 60 seconds to absorb magnetic beads. Collect the supernatant EB to a new Lobind tube and mark as the bacterial DNA product.

Extracted bacterial DNA product should be stored at –20°C and can be used for further analysis by downstream application, such as real-time PCR and/or next-generation sequencing.



9. WARNINGS and PRECAUTIONS ⚠

** The kit shall only be handled by educated personal in a laboratory environment!
** Handle reagents with special care. Avoid any contact with reagents. In case of contact, flush eyes or skin immediately with a large amount of water.
** Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions

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