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

λ Phage Genome Kit

Product Number: DNK2201

Shipping and Storage

1. Add 1ml of Buffer LS to RNaseA and DNaseI tubes respectively, blow and mix well, fully dissolve RNase A and DNase I, and then pack them into -20℃ freezer according to the amount used each time. The shelf life is 6 months.
2. When the Buffer LB is at low temperature, precipitation and precipitation may occur. It can be dissolved again in a water bath at 37℃ for a few minutes to help restore clarity and transparency. After cooling to room temperature, it can be used.
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.

Component

Component	Storage	DNK2201 50 Preps	DNK2202 100 Preps
RNase A	-20℃	20 mg	20 mg×2
DNase I	-20℃	50 mg	50 mg×2
Buffer BP	RT	100 ml	100ml×2
Buffer LS	RT	30 ml	60 ml
Buffer LP	RT	5 ml	10 ml
Buffer LB	RT	20 ml	40 ml
Buffer WB	RT	15 ml	25 ml
Buffer EB	RT	10 ml	15 ml
Adsorption column AC	RT	50	100
Collection tube (2ml)	RT	50	100

Description

λ Phage vectors are widely used for library screening, and the purpose is to clone and cultivate a large number of bacterial particles that need to be extracted λ Perform subsequent work such as sequencing using bacteriophage DNA. λ The supernatant of phage lysate culture after centrifugation is first digested with a mixture of RNase A/DNase I enzymes to remove residual host bacterial DNA/RNA, and then precipitated to collect phages. The phages are lysed by SDS, and the remaining fragments are removed by precipitation centrifugation. The incoming bacteriophage DNA in the supernatant of the lysate is selectively adsorbed onto the silica matrix membrane in a highly dissociated salt state, followed by a series of rapid rinsing centrifugation steps to remove impurities such as salt, cellular metabolites, and proteins. Finally, the low salt Buffer EB washes the incoming bacteriophage DNA off the silica matrix membrane.

Features

1. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
2. Time saving and simple, it can be used for the extraction of liquid culture lysates and solid culture plates. The operation of a single sample can generally be completed within 1.5 hours.
3. High yield, typical yield of 10ml λphage lysate culture supernatant can extract about 10μg λphage DNA.
4. Multiple column washes ensure high purity, with a typical OD260/0D280 ratio of 1.7 to 1.9. It can be directly used for enzyme digestion and sequencing.

Application

Suitable for fast λphage DNA

Note

1. Use a freeze centrifuge with a speed of up to 13.000rpm.
2. Preheat the required water bath to 37℃ for later use before starting the experiment.
3. We need to provide our own chloroform and 20% SDS.
4. Buffer LB contains irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.

Protocol(Please read the note before the experiment)

Example of extracting bacterial culture supernatant from 10 ml phage infection:

Tips:1)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!

2)Pre cool the Buffer BP on ice.

1. Centrifuge 10000g (approximately 12000rpm) of the liquid culture infected with λphages treated with 0.5% chloroform at 4℃ for 10 minutes to remove cell debris and residue.

   The rotation speed should not be too high and the time should not be too long, otherwise the bacteriophage may precipitate together with the fragments, reducing production.
2. Take 10ml of supernatant, add 20μl of RNase and 20μl of DNase, mix well, and incubate at 37℃ for 30 minutes.

   The amount of residual RNA/DNA in the culture supernatant of each phage varies due to different growth and lysis conditions. Excessive digestion of RNase/DNA may reduce yield; Incomplete digestion may result in undigested DNA/RNA and cell debris adhering to some phages, reducing production or ultimately contaminating host bacterial DNA. Therefore, the dosage and digestion time should be adjusted appropriately according to the actual situation.
3. Add 2ml of pre cooled buffer BP with ice, mix gently and thoroughly before cooling on ice (the culture plate lysate must be left on ice for 30 minutes).
4. Centrifuge at 4℃ for 10 minutes at 10000g (12000rpm), discard the supernatant, and dry for 1 minute. The precipitated bacteriophage appears as a translucent or slightly white precipitate.
5. Add 500μl of Buffer LS, blow and resuspend the bacteriophage, add 100μl of 20% SDS, gently invert and mix 4-6 times, incubate at 70℃ for 10 minutes, and then cool on ice.
6. Add 100μl Buffer LP, immediately gently invert and mix 4-6 times, centrifuge at 4 ℃ for 10 minutes at a maximum speed of 13000g.
7. Carefully transfer the supernatant into a new centrifuge tube, add 350μl Buffer LB, and gently vortex and mix well.
8. Add the above mixture to an adsorption column AC, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid in the collection tube.

   The adsorption column can only accommodate up to about 700μl of mixture at a time, so it is necessary to load the mixture into the adsorption column in stages and repeat step 8.
9. Add 700μl of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
10. **Optional steps:** Repeat step 9 once.
11. 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.
12. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100μl of Buffer EB (pre heated in a 50 ℃ water bath) to the middle of the adsorption membrane. Let it stand at room temperature for 1 minute and centrifuge at 12000 rpm for 1 minute. If you want to obtain a large amount of DNA, you can add the obtained solution back to the centrifuge adsorption column and centrifuge at 12000 rpm 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 40µl. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.

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