DATA SHEET

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Yeast DNA Preparation Kit

Spin column based genomic DNA purification from yeast

Cat. Nº.	Amount
☐ DPK-110S	50 preparations
■ DPK-110L	250 preparations

Shipping:
Shipped at ambient temperature

Storage Conditions:
Store at ambient temperature (except Rnase, Proteinase K and Lyticase store at - 20 °C)

Shelf life:
12 months

Description:
The spin column based Yeast DNA Preparation Kit is designed for rapid and high purity isolation of genomic DNA from yeast cells. The spin column based method completely removes PCR inhibitors such as divalent cations and proteins resulting in a high purity preparation of genomic DNA. There is no use of phenol or chloroform, handling is safe and does not produce any harmful waste. Column based genomic DNA purification kits yield up to 30 µg DNA sized from 200 bp to 50 kb per preparation.

Applications:
The obtained DNA is suitable for a variety of applications, including real-time PCR, southern blot analysis, genotyping and discovery or validation of SNP/SSR markers.

Kit Contents:

- Cell Resuspension Solution
- Lyticase (before use, solve in Lyticase Suspension Solution to obtain a final concentration of 2.5 units/µl) - store at -20 °C
- Lyticase Suspension Solution
- Cell Lysis Solution
- RNase A (before use, solve in double distilled water to obtain a final concentration of 50 mg/ml) - store at -20 °C
- Proteinase K (before use, add double distilled water as indicated on the bottle - store at -20 °C)
- Washing Buffer (before use, add 96-99 % Ethanol as indicated on the bottle)
- Binding buffer
- Activation buffer
- Elution Buffer
- Spin Columns and 2 ml Collection Tubes

Additional Materials Required:

- Double distilled water
- Ethanol 96-99 %
- 1.5 or 2.0 mL microtubes

For S pack (50 preps): Before start, add 500 µl dd-water to the Proteinase K tube, 70 µl Lyticase Buffer to the Lyticase tube, 150 µl dd-water to the RNase A tube and 44 ml 96-99 % Ethanol (not included in the kit) to the Washing Buffer bottle.

For L pack (250 preps): Before start, add 500 µl dd-water to each Proteinase K tube, 70 µl Lyticase Buffer to each Lyticase tube, 150 µl dd-water to each RNase A tube and 208 ml 96-99 % Ethanol (not included in the kit) to Washing Buffer bottle.

Buffer	DPK-110S 50 preps	DPK-110L 250 preps
Cell Ressuspension solution	5,5 ml	27 ml
Lytcase (2,5 units/µL)	175 units	5 x 175 units
Lyticase suspension solution	100 µl	500µl
Lysis buffer	16 ml	80 ml
Binding buffer	16 ml	80 ml
Rnase A (50 mg/mL)	7,5 mg	5 x 7,5 mg
Proteinase K (10 mg/ml)	5 mg	5 x 5 mg
Activation buffer	6 ml	27,5 ml
Washing buffer	add 44 ml EtOH	add 208 ml EtOH
Elution buffer	3 ml	13 ml

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It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purify. A maximum amount of 1 - 2 x 10^7 yeast cells can generally be processed. Overnight cultured yeast cells can be processed. Cell pellets can be stored at -70 °C for several months.

1. Cell Lysis:

- Transfer 0,5 ml of cultured cells into a 1.5 ml microtube
- To harvest the cells centrifuge at 8,000 g for 1 min and discard the supernatant.
- Proceed immediately
- Add 100 µl of Cell Ressuspension Solution
- Add 1 µl of Lyticase Suspension Solution to the cell pellet
- Vortex vigorously for 10 sec
- Incubate for 15 min at 37 °C
- Centrifuge at 10,000 g for 1 min
- Discard supernatant
- Add 300 µl Lysis Buffer and 2 µl RNase A to cell pellet
- Vortex vigorously for 10 sec

- Add 8 µl Proteinase K to the cell lysate and mix by pipetting
- Incubate at 60 °C for 10 min and cool down on ice for 5 min
- Add 300 µl Binding Buffer to the cell lysate
- Vortex briefly
- Place the tube on ice for 5 min
- Centrifuge for 5 min at 10,000 g

2. Column Activation [optional]:

- Place a spin column into a 2 ml collection tube
- Add 100 µl Activation Buffer into the Spin Column
- Centrifuge at 10,000 g for 30 sec and immediately proceed to next step
- Discard the flow-through

3. Column Loading:

- Pipet the supernatant directly into the spin column
- Centrifuge for 1 min at 10,000 g
- Discard the flow-through

4. Primary Washing:

- Add 500 µl Washing Buffer into spin column
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through

5. Secondary Washing:

- Add 500 µl Washing Buffer into the spin column
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through
- Centrifuge again at 10,000 g for 1 min to remove residual Washing Buffer
- Discard the 2 ml wash tube and place the column in the elution tube

6. Elution of DNA:

- Add 40-50 µl Elution Buffer into the center of the column
- Incubate at room temperature for 1 min
- Centrifuge at 10,000 g for 2 min
- Store DNA at 4 °C or -20 °C