High-Q™ Yeast Genomic DNA Purification Kit

Ordering info

TBK0180, 3 reactions (sample)

TBK0181, 50 reactions

TBK0182, 200 reactions

Description

High-Q™ Yeast Genomic DNA Purification Kit is an optimized kit with double lysis procedure to obtain high molecular weight genomic DNA from yeast culture. The procedure includes enzymatic and mechanical lysis with beads to break down the thick cell wall of yeast. Genomic DNA released is adsorbed in silica spin columns and washed to remove proteins, salts and inhibitors.

Features

- High yield and purity, 3-15 µg, A_{260}/A_{280} = 1.8 ± 0.2; A_{260}/A_{230} = 2.0 ± 0.2.
- Safe, method does not require phenol extraction.
- Easy protocol.
- Cost-effective.

Applications

DNA obtained is suitable for downstream molecular biology applications such as PCR, enzymatic digestion for cloning or Southern, genotyping, etc.

Quality Control

DNA isolation from 1.5 mL yeast culture is checked by: integrity (agarose gel electrophoresis), quantity and quality (A260/280= 1.8 ± 0.2; A260/230= 2.0 ± 0.2).

Kit Components

Components	TBK0181	TBK0182
High-Q™ Spin Column with Collection Tubes	50	200
Beads-Tube	50 x 0.5 g	200 x 0.5 g
Proteinase K	30 mg	3 x 30 mg
Proteinase K Resuspension Buffer	1.5 mL	3 x 1.5 mL
BYeast-1 Buffer [e]	12 mL	45 mL
BYeast-2 Buffer	12 mL	45 mL
BYeast-3 Buffer	12 mL[a]	48 mL[b]
WB2 Buffer	12 mL[c]	45 mL[d]
Elution Buffer	15 mL	25 mL

Order Info Kit Components: *High-Q™ Spin Column with Collection Tubes (TBM0010) | Beads-Tube (TBR0126) | Proteinase K (TBZ0303) | Proteinase K Resuspension Buffer (TBB0546) | BYeast-1 Buffer (TBB0517) | BYeast-2 Buffer (TBB0518) | BYeast-3 Buffer (TBB0511) | WB2 Buffer (TBB0512) | Elution Buffer (TBB0510).*

Before its use:

[a] Add 18 mL absolute ethanol and mix well.

[b] Add 72 mL absolute ethanol and mix well.

[c] Add 48 mL absolute ethanol and mix well.

[d] Add 180 mL absolute ethanol and mix well.

[e] Add 10 µL β-Mercaptoethanol per 1 mL.

Storage

Store the kit at 25°C.
Store Proteinase K at -20°C.

Material required (not supplied)

- RNase A (CAS 9001-99-4) (*Optional*)
- Ethanol (CAS 64-17-5).
- Isopropanol (CAS 67-63-0).

PROTOCOL

1. Transfer **1.5 mL early exponential phase yeast culture** (~1-2 x 10^7 cells) to 1.5 mL tube.
   For S. cerevisiae WT grown in YPD, 1 OD at 600nm is around 0.5-2 10^7 cell/ mL[1].
2. Add **200 µL BYeast-1 Buffer** and **200 µL BYeast-2 Buffer**, homogenize using a pipette. Transfer the sample mixture to a **Beads-Tube.**
3. Homogenize by continuously horizontal shaking for 15 minutes.
4. Add **20 µL Proteinase K (20 mg/mL)**.
5. Mix briefly by vortex and incubate at 60°C, 30 minutes.
6. Centrifuge at 13,000 g, 2 minutes and transfer the supernatant to a clean 1.5 mL tube.

   ***Optional**, if RNA-free preparation is required: Add **20 µL RNase (10 mg/mL).** Incubate 5 minutes at 37°C.*
7. Add **300 µL Isopropanol** and mix by inversion 3-4 times.
8. Transfer the mixture to a High-Q™ Spin Column placed into a Collection Tube.
9. Centrifuge at 10,000 g, 1 minute. Discard the collection tube and place the High-Q™ Spin Column into the Collection Tube.
10. Add **500 µL BYeast-2 Buffer**.
11. Centrifuge at 10,000 g, 1 minute. Discard the flow-through and place back the High-Q™ Spin Column into the Collection Tube.
12. Add **500 µL WB2 Buffer.**
13. Centrifuge at 10,000 g, 1 minute. Discard the flow-through and place back the High-Q™ Spin Column into the Collection Tube. Repeat step 12 and 13.
14. To dry Spin Column, centrifuge again at 13,000 g for 2 minutes.
15. Place the High-Q™ Spin Column into a clean 1.5 mL Tube.
16. Add **50-100 µL prewarmed Elution Buffer** or **Water, Molecular Biology Grade** (pre-warmed at 70°C).
17. Incubate at room temperature, 2 minutes.
18. Centrifuge at 13,000 g for 1 minute.
19. Check DNA quality on agarose electrophoresis gel and quantity by spectrophotometry.
20. Store at -20°C.

References: 1. DeNobel, JG, Kils FM, Priem J, Munnik T, van den Ende H (1990) Yeast 6:491-499.