

# DATA SHEET

Version: 2  
Revision date: 17/04/2023

## 1. Identification

Product name	HigherPurity™ Yeast Genomic DNA Isolation Kit
Cat. No	AN0080 (50 reactions)
Cat. No	AN0081 (100 reactions)
Cat. No	AN0081-XL (250 reactions)

## 2. Description

HigherPurity™ Yeast Genomic DNA Isolation Kit is a simple and rapid method for high-quality genomic DNA purification from yeast. The kit is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts. The Kit combines the power of spin column technology with the lyticase and alkaline-SDS lysis of yeast cells. The cell wall of yeast cells are rapidly and efficiently lysed enzymatically by lyticase. The sample DNA is then bound to the surface of a Spin Filter membrane and washed and the bound DNA is then desorbed from the surface of the Minispin column. The inhibitors of the downstream PCR will be removed by utilizing the DNA binding column and the buffers system in this kit.

## 3. Kit Components

Item	AN0080	AN0081	AN0081-XL
Buffer BLL	30 ml	60 ml	150ml
Buffer BLY	15 ml	30 ml	75 ml
Beads Tube	50	100	250
Wash Buffer 1 * (WB1)	22 ml	44 ml	110 ml
Wash Buffer 2 * (WB2)	10 ml	20 ml	50 ml
Elution Buffer (EB)	15 ml	30 ml	50 ml
Proteinase K **	20 mg	2x20 mg	5x20 mg
Lyticase solution	5x500 µl	10x500 µl	25x500 µl
MiniSpin columns	50	100	250
Collection tube (2mL)	100	200	500

### Note

\*Add the volume ethanol (96%-100%) specified [Not included] to WB1 and WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

\*\*Dissolve Proteinase K in water to obtain a 20 mg/mL stock solution. **The Proteinase K solution can be stored for several days at 2-8 °C.** For longer-term storage, the unused portion of the solution may be stored in aliquots at -20 °C until needed.

## 4. Features

- **Safe:** no phenol-chloroform extraction.
- **Efficient.**
- **Ready to use** genomic DNA, in all molecular biology applications.

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### 5. Storage specifications

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K at 4°C. Lyticase solution should be stored at -20°C. All other kit components can be stored at room temperature.

**Important!** BLL Buffer containing 14 mM of  $\beta$ -mercaptoethanol is hazardous to human health. Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

### 6. Applications

High molecular weight genomic DNA purified with the kit is suitable for direct use in all common molecular biology applications: PCR, cloning, DNA sequencing, Southern blot analysis, etc.

### 7. PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development and is not suitable for administration to humans or animals. Please refer to [www.canvaxbiotech.com](http://www.canvaxbiotech.com) for the Material Safety Data Sheet of the product.

## DETAILED PROTOCOL

1. Transfer yeast culture (maximum  $5 \times 10^7$  cells) to 1.5 ml microcentrifuge tube (not provided) and centrifuge 10 min at  $5000 \times g$ . Discard supernatant.

💡 Harvest yeast during early log phase of growth.

2. Resuspend the cell pellet in **600 µl** of **Buffer BLL**.

3. Add **50 µl** of **lyticase solution** and incubate at  $30^\circ\text{C}$  for 30 min.

4. **[Optional step]** If RNA-free genomic DNA is required, add 8 µl of 50 mg/ml RNase A (not provided), mix by shaking vigorously and incubate for 5 minutes at room temperature.

5. Centrifuge at  $5000 \times g$  for 5 min. Remove supernatant by pipetting.

6. Add **200 µl** of **Buffer BLY** and continue to homogenize the sample by pipetting.

7. Transfer the sample mixture to a **beads tube**. Mix well by vortexing vigorously for 5 minutes.

8. Add **15 µl** of **Proteinase K** (20mg/ml), mix by shaking vigorously, and incubate at  $56^\circ\text{C}$  for 30 minutes. During incubation, invert the tube regularly.

9. Centrifuge 1 min at  $5000 \times g$  and transfer supernatant to a new 1.5 ml microcentrifuge tube. (not provided).

10. Add **200 µl Absolute Ethanol** (not provided) and mix immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. In case precipitate appears, break it up by pipetting.

11. Place the **MiniSpin column** in a **collection tube** and transfer the sample mixture (including and precipitate if present) to the column.

12. Centrifuge at  $6000 \times g$  for 2 minutes. Discard the collection tube containing the flow-through and place the **MiniSpin column** in a new **collection tube**.

13. Add **500 µl** of **Buffer WB1** and centrifuge at  $16000 \times g$  for 30 seconds. Discard the flow-through and place the **MiniSpin column** back in the **collection tube**.

14. Add **750 µl** of **Buffer WB2** and centrifuge at  $16000 \times g$  for 1 minute.

15. Discard the flow-through and place the **MiniSpin column** back in the **collection tube** and centrifuge for another 3 minutes at  $16000 \times g$  to dry the matrix of the column.

16. Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet **100 µl** pre-heated **Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature 5 minutes.

17. Centrifuge for 1 minute at  $16000 \times g$  to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at  $-20^\circ\text{C}$ .

