# Data sheet

## HigherPurity™ Yeast Genomic DNA Isolation Kit

**Cat. No: AN080 (50 reactions)**
**Cat. No: AN081 (100 reactions)**

### 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.

### Features

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

### 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.

| Kit Components | (Reactions) | |
|---|---|---|
| **Item** | **50** | **100** |
| Buffer BLL | 30 ml | 60 ml |
| Buffer BLY | 15 ml | 30 ml |
| Beads Tube | 50 | 100 |
| Wash Buffer 1 *(WB1) | 22 ml | 44 ml |
| Wash Buffer 2 * (WB2) | 10 ml | 20 ml |
| Elution Buffer (EB) | 30 ml | 30 ml |
| Proteinase K ** | 20 mg | 2 x 20 mg |
| Lyticase solution | 5x500µl | 10x500µl |
| MiniSpin columns | 50 | 100 |
| Collection tube (2mL) | 100 | 200 |

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.

### Kit Storage:

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K at 4ºC. Lyticase solution should be stored at -20ºC. All other kit components can be stored at room temperature. The kit components are stable for 1 year, if stored properly.

BLL Buffer containing 14 mM of ß-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.

*(Continued on reverse side)*

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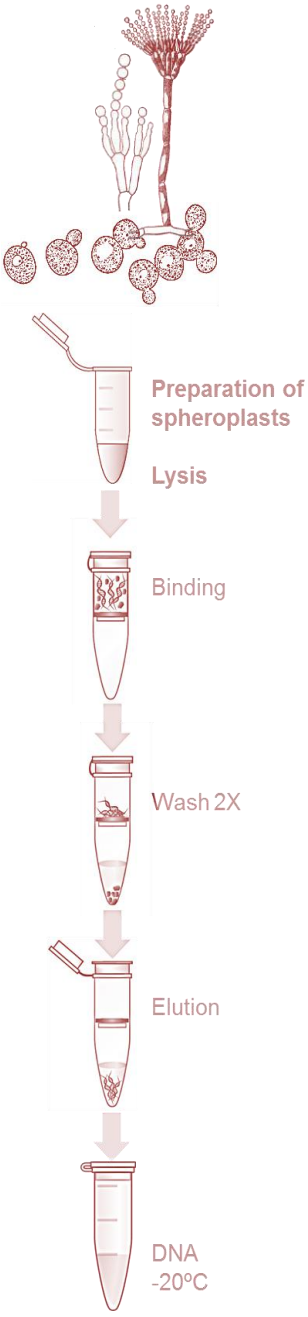
# DETAILED PROTOCOL

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

   *Harvest yeast during early log phase of growth.*
2. Resuspend the cell pellet in **600 µl** of **Buffer BLL**.
3. Add **50µl** of **lyticase solution** and incubate at 30 °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 x *g* for 5 min. Remove supernatant by pipetting.
6. Add **200µl** 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 **15µl** of **Proteinase K** (20mg/ml), mix by shaking vigorously, and incubate at 56ºC for 30 minutes. During incubation, invert the tube regularly.
9. Centrifuge 1 min at 5000 x *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 any precipitate if present) to the column.
12. Centrifuge at 6000 x *g* for 2 minutes. Discard the collection tube containing the flow-through and place the **MiniSpin column** in a new **collection tube**.
13. Add **500µl** of **Buffer WB1** and centrifuge at 16000 x *g* for 30 seconds. Discard the flow-through and place the **MiniSpin column** back in the **collection tube**.
14. Add **750µl** of **Buffer WB2** and centrifuge at 16000 x *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 x *g* to dry the matrix of the column.
16. Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet **100µl** pre-heated **Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature 5 minutes.

    ***Notes:*** *Standard elution volume is 100µl. To increase concentration, elute with 30-50µl. To increase yield, elute with 200µl.*
17. Centrifuge for 1 minute at 16000 x *g* to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20ºC.




**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 for the Material Safety Data Sheet of the product.

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