# Zymo Quick-DNA Midiprep plus

**Recovery.**  Up to 1**25** μg DNA > 50 kB

**Sample Sources** 1-30 x 106 cells

**DNA Elution Buffer:** Supplied buffer = 10 mM Tris-HCl (pH 8.5), 0.1 mM EDTA

If next step will be hydrolysis of the DNA, then elute in EDTA – free buffer, eg 10 mM Tris-HCl (pH 8.0)

**Prepare Proteinase K buffer**

Add 1040 μL Proteinase K Storage Buffer to 20 mg proteinase K. Store at -20 °C.

## Protocol.

### 1. Lyse cells

1. 1 x 106  cells suspended inPBS. (Low ionic strength buffer)
2. Mix with equal volume of Biofluid and Cell Buffer (Red)
3. Mix with Proteinase K
4. Vortex 15 s
5. Incubate 55°C for 40 – 120 min

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Cells | 1 x 106 | 5 x 106 | 10 x 106 | 20 x 106 | 30 x 106 |
| a | Suspension buffer | 0.5 mL | 1 mL | 1.25 mL | 1.5 mL | 2 mL |
| b | BioFluid and Cell Buffer | 0.5 mL | 1 mL | 1.25 mL | 1.5 mL | 2 mL |
| c | proteinase K | 20 μL | 40 μL | 50 μL | 60 μL | 80 μL |
| e | Incubation time 55 C | 40 min | 60 min | 80 min | 100 min | 120 min |
|  | Genomic Lysis Buffer | 1 mL | 2 mL | 2.5 mL | 3 mL | 4 mL |

**2. Load sample on column.**

1. Add 1 volume of **Genomic Lysis Buffer**
2. Mix thoroughly (vortex 10-15 s)
3. Transfer sample to Zymo-Spin V-E Column/Reservoir inserted in a 50 ml conical tube
4. Centrifuge 1,000 g for 5 min , Discard flow through

**3. Wash off impurities.**

1. Add **9 mL** **DNA Pre-Wash** buffer
2. Centrifuge 1,000 g for 5 min, Discard flow through
3. Add **7 m**L **g-DNA Wash** buffer
4. Centrifuge 1,000 g for 5 min, Discard flow through.
5. Remove and discard the Reservoir,
6. Place the Zymo-Spin™ V-E Column into a Collection Tube.
7. Centrifuge at 12,000 x g for 60 s in a microcentrifuge tube to remove residual wash buffer from the column
8. Add **200** μL **g-DNA Wash** buffer
9. Centrifuge at 12,000 x g for 60 s, discard the flow through

**4. Elute off DNA**

1. Transfer column to 1.5 mL centrifuge tube
2. Add ≥ **50** μL **DNA Elution Buffer**, warmed to 60-70°C, directly to column matrix
3. Incubate **5** min
4. Centrifuge 12,000 x g for 60 s
5. **This will be the DNA**
6. Measure with nanodrop.