

# PUREGENE® DNA Purification Kit

## DNA Purification from Buccal Cells in Mouthwash

Expected DNA Yield Range: 4 µg - 40 µg, Mean: 16 µg

Estimated Time to Process Four Samples: 2hrs, 45mins – 3hrs

### Buccal Cell Collection and Cell Lysis

1. Dispense 10 ml Original Mint Scope Mouthwash into a 50 ml tube.
  2. Collect buccal cells by swishing orally the 10 ml of mouthwash 10-20 times and spitting back into the 50 ml tube.
- Notes i ) For best results, wait at least one hour after eating or drinking to collect buccal cells.  
ii ) Buccal cells are stable at room temperature for at least 7 days in the mouthwash solution.
3. Centrifuge at 2,000 x g for 10 minutes to concentrate the cells. Immediately pour off supernatant leaving behind 100 µl of residual liquid. *Loose pellets will result if samples sit too long after centrifugation.*
  4. Vortex vigorously for 5 seconds to resuspend the cells in the residual supernatant. This greatly facilitates cell lysis in Step 5 below.
  5. Add 3 ml **Cell Lysis Solution** to the resuspended cells and vortex 5 seconds at medium speed to maximize contact between cells and Cell Lysis Solution.
  6. Add 15 µl of **Proteinase K** (20 mg/ml) and incubate lysate at 55°C for 1 hour.

### RNase Treatment (Optional)

1. Add 15 µl **RNase A Solution** to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37°C for 15 minutes

### Protein Precipitation

1. Cool sample to room temperature.
2. Add 1 ml **Protein Precipitation Solution** to the cell lysate.
3. Vortex samples at high speed for 20 seconds to mix the **Protein Precipitation Solution** uniformly with the lysate.
4. Place tube in an ice bath for 10 minutes to ensure a tight pellet in Step 5 below.
5. Centrifuge at 2,000 x g for 10 minutes. The precipitated proteins should form a tight, green pellet.

### DNA Precipitation

1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 50 or 15 ml tube containing 3 ml **100% Isopropanol** (2-propanol) and 5 µl **Glycogen Solution** (20 mg/ml).
2. Mix the sample by inverting gently 50 times and keep tube at room temperature for at least 5 minutes.
3. Centrifuge at 2,000 x g for 10 minutes. The DNA may or may not be visible as a small white pellet, depending on yield.
4. Pour off the supernatant and drain tube briefly on clean absorbent paper. Add 3 ml **70% Ethanol** and invert the tube several times to wash the DNA pellet.
5. Centrifuge at 2,000 x g for 3 minutes. Carefully pour off the Ethanol.
6. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes

### DNA Hydration

1. Add 200 µl of **DNA Hydration Solution** (200 µl will give a concentration of 100 ng/µl if the total yield is 20 µg of DNA).
2. Rehydrate DNA by incubating at 65°C for 1 hour and overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
3. For storage, vortex sample briefly, pulse spin, and transfer to a 1.5 ml tube. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.