

User-Developed Protocol

Isolation of fungal DNA from serum using the Roche High Pure PCR Template Preparation Kit

Procedure for the isolation of nucleic acids from $\geq 500 \mu\text{L}$ serum



Before starting, warm up the Elution Buffer to 70°C

- 1) Add a minimum of $500 \mu\text{L}$ of serum to $400 \mu\text{L}$ of Binding Buffer and $80 \mu\text{L}$ of reconstituted Proteinase K, mix immediately and incubate at 70°C for 10 minutes.
- 2) Add $200 \mu\text{L}$ of Isopropanol and mix well.
- 3) Apply the entire volume (by repeat addition) to the spin column and centrifuge at $8000 \times g$ for 1 minute for each addition. Discard the flow-through.
- 4) Apply $500 \mu\text{L}$ of Inhibition Removal Buffer to the upper reservoir of the spin filter tube and centrifuge at $8000 \times g$ for 1 minute. Discard the flow-through.
- 5) Apply $500 \mu\text{L}$ of Wash Buffer to the upper reservoir of the spin filter tube and centrifuge at $8000 \times g$ for 1 minute. Discard the flow-through.
- 6) Repeat step 5.
- 7) Centrifuge at maximum speed to remove any last traces of Wash Buffer.
- 8) Add $65 \mu\text{L}$ of pre-warmed (70°C) Elution Buffer to the upper reservoir of the spin filter tube and centrifuge at $8000 \times g$ for 1 minute.
- 9) Transfer the eluate to sterile microtube.
- 10) Store the eluate at -80°C until testing.

References

This UDP was kindly supplied by P. Lewis White (personal communication)

Roche High Pure PCR Template Preparation Kit Cat. No. 11 796 828 001

UDP 3: Isolation of fungal DNA from serum using the Roche High Pure PCR Template Preparation Kit

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