Huwel Fungal DNA Extraction Kit

HL-FDX-100: 100 Exts

RUO

PI/HLFDX-01

Intended use

Huwel Fungal DNA Extraction kit is used to extract Fungal DNA from Clinical samples by Spin column method. The extracted Fungal DNA is used as sample for diagnostic determination of Fungal Infection by Real-Time PCR Amplification method.

Background Information

Huwel Fungal DNA Extraction Kit provides a simple, nontoxic method for efficiently isolating high-molecular-weight DNA (Genomic DNA) from tissue and other body fluids. Depending on the starting material, the entire extraction takes only 30 minutes to complete and does not require phenol or chloroform. DNA isolated with the Huwel Fungal DNA Extraction Kit is free from contaminants and may be used directly for restriction digestion, cloning, PCR amplification, and other DNA analysis techniques.

Kit components:

Contents	Description	100 Exts (HL-FDX-100)
Huwel Solubilization Buffer	RBC Lysis solution	1 x 50 mL
Proteinase K	Lyophilized	100 mg
Quick Lysis Buffer	Lysis Buffer	1 x 50 mL
Huwel Wash buffer-1	Wash buffer-1	1 x 50 mL
Huwel Wash buffer-2	Wash buffer-2	2 x 50 mL
Huwel Elution Buffer	Elution buffer	1 x 20 mL
Spin columns	NA	100 no.s
Collection tubes	NA	200 no.s

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Note: Huwel Wash Buffer 1 and Huwel Wash Buffer 2 are provided with Ethanol. No need to add additional Ethanol.

Note: Reconstitute the Lyophilized Proteinase K in 5 mL of Huwel Elution Buffer. Prepare small aliquots according to the user requirement and store at -15°C to -25°C; stable for 12 months

Storage:

Component	Storage Conditions
Huwel Solubilization Buffer	2-8 °C upon Arrival
Proteinase K	-15°C to -25°C
Quick Lysis Buffer	Room Temperature
Huwel Wash Buffer-1	Room Temperature
Huwel Wash Buffer-2	Room Temperature
Huwel Elution Buffer	Room Temperature
Spin Columns	Room Temperature
Collection Tubes	Room Temperature

Procedure:

Protocol for DNA Extraction from Fluids (CSF and BAL Fluid) :

1. Take approximately 500 µL-1 mL of sample in the microcentrifuge tube and spin at 10,000 rpm for 1min.
2. Discard the supernatant, leaving small amount of liquid (approx. 100µL).
3. Resuspend the liquid in 300 µL Huwel Solubilization buffer add 20 µL Proteinase-K and vortex the tube for 5 sec.
4. Incubate at 65°C for 10 min followed by centrifugation at 5000 rpm for 10 min.
5. Discard the supernatant, leaving small amount of liquid (approx. 100µL).
6. Add 500 µL Quick Lysis Buffer and incubate at room temperature for 20min.
7. After incubation, Load 700µL of sample lysate into Huwel spincolumn.
8. Centrifuge at 8000 rpm for 1 min followed by discarding the flowthrough and collection tube.
9. Repeat step 8 for total lysate.
10. Insert the spin column in to new collection tube and add 500 µL of Huwel Wash Buffer-1.
11. Centrifuge at 8000 rpm for 1 min followed by discarding the flowthrough and collection tube.

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12. Change the collection tube, add 500 µL of Huwel Wash Buffer-2.
13. Centrifuge at 8000 rpm for 1 min followed by discarding the flow through.
14. Re-Use the same collection tube for empty spin.
15. Centrifuge at 12000 rpm for 1 min and discard the collection tube.
16. Insert the Spin column in fresh 1.5 mL microcentrifuge tube and add 100 µL of Huwel Elution Buffer.
17. Incubate the column at RT for 5 mins.
18. Centrifuge at 8000 rpm for 1 min.
19. Store the collected DNA at-20°C until use.

Protocol for DNA Extraction from Blood samples:

1. Take 15 mL Huwel Solubilization buffer add 20 µL Proteinase K and add 1 mL of blood sample and vortex for 20 seconds.
2. Incubate on ice for 10-15 min.
3. Centrifuge at 3000 rpm for 10 min.
4. Discard the supernatant.
5. Repeat step 1 to 4 until the red mat color disappears.
6. Then continue with the same procedure as detailed in page no.2-3, Steps 6 to 18 of Protocol for DNA Extraction from Fluids.

Protocol for DNA Extraction from Tissue/Biopsy samples:

1. Cut tissue into small pieces to ensure rapid lysis and high yields.
2. Weigh the appropriate tissue amount (between 15 to 25 mg) and place in a 1.5 mL microfuge tube.

   Note: Using more than the recommended amounts will not lead to better yields and/or purity. If using more than recommended is required, split the sample into 2 or more preps.
3. Add 1 mL Huwel Solubilization Buffer and 20 µL Proteinase K and mix thoroughly by vortexing for 20 sec.
4. Incubate on ice for 10-15 min.
5. Centrifuge at 3000 rpm for 10 min.
6. Discard the supernatant.
7. Repeat step 1 to 6 until the red mat color in the tissue disappears.
8. Then continue with the same procedure as detailed in page no.2-3, Steps 6 to 18 of Protocol for DNA Extraction from Fluids.

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Protocol for DNA Extraction from Culture from slants and culture plates:

1. Take loopful of culture using microtip or sterilized inoculation loop.
2. Resuspend in 200 µL Quick Lysis buffer and continue with the protocol/procedure as detailed in page no.2-3, Steps 6 to 18 of Protocol for DNA Extraction from Fluids.

Protocol for DNA Extraction from Liquid Culture

1. Take 1 mL of Liquid fungal culture in the microcentrifuge tube, spin at 10,000 rpm for 1 min.
2. Discard the supernatant. Wash the pellet twice with sterile Molecular Grade Water.
3. Discard the supernatant. leaving small amount of liquid (approx 100µL).
4. Then follow the same procedure as detailed in page no.2-3, Steps 6 to 18 of Protocol for DNA Extraction from Fluids.

Troubleshoot

The most common problem for PCR is PCR inhibitors recovered with the nucleic acid. This can usually be overcome by either using 10-fold less nucleic acid in the PCR, or by using less tissue as starting material in the prep. Another problem is degraded nucleic acid. Degradation is usually due to a long lag time between harvesting the specimen and starting the prep, or due to repeated freeze-thaw of frozen tissue. To avoid degradation, disrupt the tissue as soon as possible after harvesting or store it in a reagent designed to preserve nucleic acid in solid tissue. Alternatively, harvested tissue may be snap-frozen and stored at -70°C. Storage at-20°C may result in gradual decline in nucleic acid integrity. Note however that degraded nucleic acid is usually adequate for use as template in PCR, especially for amplicons shorter than ~500bp.

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Quality management system is certified in compliance with the requirements of ISO 9001:2015 and ISO 13485:2016