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Zymo Duet DNA/RNA MiniPrep Plus Extractions

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We use this protocol and it's working

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Abstract

Attached are detailed instructions for following the kit guidelines for the Zymo Duet DNA/RNA MiniPrep Plus.

_D7003_ZR-Duet_DNA-RNA_MiniPr…

Guidelines

Follow kit guidelines for best practices.

_D7003_ZR-Duet_DNA-RNA_MiniPr…

Materials

MATERIALS

ZR-Duet™ DNA/RNA MiniPrep Plus **Zymo Research Catalog #**D7003

	Reagent in Kit	Quantity	Storage Temperature	Notes on Preparation
	DNA/RNA Lysis Buffer	50 ml	Room Temp.	
	DNA/RNA Prep Buffer	50 ml	Room Temp	
	DNA/RNA Wash Buffer (concentrate)	24 ml	Room Temp	Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml DNA/RNA Wash Buffer concentrate before use
	DNase/RNase-Free Water	10 ml	Room Temp	
	DNase I (lyophilized)	1	Room Temp (lyophilized), -20 C (reconstituted)	Prior to use, reconstitute the lyophilized DNase I with 275 µl DNase/RNase-Free Water. Mix by inversion. Store aliquots at -20° C.
	DNA Digestion Buffer	4 ml	Room Temp	
	DNA/RNA Shield™ (2x concentrate)	25 ml	Room Temp	Dilute in DNase/RNase-free Water to obtain 1X for storage
	PK Digestion Buffer	5 ml	Room Temp	
	Proteinase K	20 mg	Room Temp (lyophilized), -20 C (reconstituted)	Prior to use, reconstitute the lyophilized Proteinase K with 1040 µl Proteinase K Storage Buffer. Vortex to dissolve. Store at -20° C
	Spin-Away™ Filters	50	Room Temp	
	Zymo-Spin™ IIICG Columns	50	Room Temp	
	Collection Tubes	3x50	Room Temp	
	Other Reagents and Equipment			
	Bead bashing tubes (ZR BashingBead™ Lysis Tubes or Omni 2.8mm ceramic bead bashing tubes or other)			
	Qiagen Tissulyser II			
	Filtered Pipette tips			
	Ethanol			
	Eppendorfs (1.5 mL)			
	Microcentrifuge			
	Eppendorf rack			

Safety warnings

Operate in an appropriate BSL level space for your sample type and suspected infectious agents.

Before start

Ensure your samples have been stored at -80C, preferably in RNA/DNA shield. Avoid freeze thaws to ensure sample integrity. Identify your sample type and follow the 'sample preparation' section for that type.

Sample Preparation for cultured isolates

1

1. Add 320 µL 1X DNA/RNA shield (2x concentrate diluted in nuclease-free water) to bead bashing tubes
2. Select colonies and add to DNA/RNA shield in bead bashing tube.
3. Tissuelyse on Qiagen Tissuelyser II 00:02:00 minutes at a frequency of 1/30 s. Alternatively, vortex at the highest frequency for 2 minutes.
4. Spin 00:01:00 at 14000RCF.
5. Add 320 µL of DNA/RNA Lysis buffer. Proceed with Nucleic Acid Purification.

Sample Preparation for liquids (ie, plasma, tracheal aspirate, CSF)

2

1. Add 1 part sample to 3 parts DNA/RNA lysis buffer (ie, 100 µL sample to 300 µL lysis buffer) in a bead bashing tube
2. Tissuelyse on Qiagen Tissuelyser II 00:01:00 minutes at a frequency of 1/30 s. Alternatively, vortex at the highest frequency for 1 minutes.
3. Spin 00:01:00 at 14000RCF. Proceed with Nucleic Acid Purification.

Sample Preparation for samples in DNA/RNA shield (1x)

3

1. If not homogenized, tissuelyse on Qiagen Tissuelyser II in bead bashing tube for desired amount of time at a frequency of 1/30 s. Alternatively, vortex at the highest frequency.
2. Add one part DNA/RNA Lysis buffer (ie 200 µL of sample in DNA/RNA shield to 200 µL of lysis buffer). Proceed with Nucleic Acid Purification.

Sample Preparation for whole blood

4

1. Add 200 µL of 2X DNA/RNA shield to each 200 µL of b2 of blood sample and mix throughly.
2. For every 400 µL of reagent/blood mixture, add 8 µL of reconstituted Proteinase K and mix thoroughly.

3. Incubate at at room temperature for 00:30:00 minutes
4. Add an equal volume of isopropanol to the reagent/blood mixture and mix by vortexing. Proceed with Nucleic Acid Purification.

Nucleic Acid Purification

5 Label a Spin-Away Filter (yellow) and the microcentrifuge tube for each sample.

6 Transfer up to 700 µL of the sample into a Spin-Away™ Filter (yellow) in a Collection Tube and centrifuge 00:00:30 seconds at 16000 RCF. If you have > 700 µL, you can reload the column and centrifuge again. Save the flow-through if you wish to proceed with RNA Purification. If not, discard flow through, and skip RNA Purification steps.

(***For whole blood ONLY***, after doing the above, discard flow-through. Add 200 µL of DNA/RNA Lysis buffer directly to the filter matrix. Let stand for 00:05:00 minutes. Then centrifuge 00:00:30 seconds at 16000 RCF. **SAVE** this flow through as your RNA is in it)

7 Proceed using the yellow column with DNA Purification sectio, and using the flow-through with the RNA purification section. After that, you will continue with the Nucleic Acid Purification for both DNA and RNA in parallel.

DNA Purification

8 DNA is bound to the yellow column. Transfer the Spin-Away™ Filter (yellow) into a new Collection Tube. Proceed with Nucleic Acid Purification.

RNA Purification

9 Transfer flow-through into Eppendorf if necessary. Add an equal volume of ethanol (95-100%) to the flow-through and mix well.

10 Transfer up to 700 µL of the sample with ethanol into a Zymo-Spin™ IIICG Column (green) in a Collection Tube and centrifuge 00:00:30 seconds at 16000 RCF. Discard the

flowthrough. Repeat by reloading the column if you have more sample remaining.

11 Wash the column with 400 µL DNA/RNA Wash Buffer and centrifuge 00:00:30 seconds at 16000 RCF. Discard the flowthrough.

12 Add 80 µl DNase I Reaction Mix (5 µL reconstituted DNAse + 75 µL DNA Digestion Buffer) directly to the column matrix. (can make a master mix of the same proportions for multiple samples)

13 Incubate the column at room temperature (20 °C - 30 °C) for 00:15:00 minutes . Proceed with Nucleic Acid Purification.

Nucleic Acid Purification

14 Add 400 µL DNA/RNA Prep Buffer to your (yellow/green) column and centrifuge 00:00:30 seconds at 16000RCF. Discard the flow-through.

15 Add 700 µL DNA/RNA Wash Buffer and centrifuge 00:00:30 seconds at 16000RCF. Discard the flow-through.

16 Add 400 µL DNA/RNA Wash Buffer and centrifuge the column for 00:02:00 minutes at 16000RCF to ensure complete removal of the wash buffer. Carefully transfer the column into clean microcentrifuge/Eppendorf tubes.

17 Add 50 µL DNase/RNase-Free Water directly to the column matrix, let stand for 00:05:00 minutes , and then centrifuge 00:00:30 seconds at 16000RCF to elute DNA and RNA from the respective column.

18 Take all 50 µL of eluted DNA/RNA in microcentrifuge tube and reload onto column. Let stand for 00:05:00 minutes, and then centrifuge 00:01:00 minute at 16000RCF to elute more DNA and RNA from the respective column.

19 Aliquot RNA into single use aliquot tubes and freeze at -80. Freeze DNA at -20 or -80.