Mag-Bind® FFPE DNA/RNA 96 Kit

Revision No: 3.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	M6955-00	M6955-01
Purifications	1 x 96	4 x 96
FDR Buffer	50 mL	180 mL
MB4 Buffer	75 mL	275 mL
RMP Buffer	25 mL	100 mL
Elution Buffer	30 mL	125 mL
Nuclease-free Water	30 mL	125 mL
DNase Digestion Buffer	25 mL	2 x 25 mL
PHM Buffer	10 mL	50 mL
Proteinase K Solution	2.2 mL	8.8 mL
Mag-Bind® DNase I	220 µL	4 x 220 µL
Mag-Bind® Particles CH	2.2 mL	10 mL

Important:
If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.

Kit includes enough reagents for the specified number or purifications plus an additional 10% overage to ensure there is sufficient volume. Please be aware that the actual number of purifications may be lower due to pre-aliquoting reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately.

Supplied by user:
- Centrifuge with swing bucket rotor capable of 2,000*g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- Incubator capable of 90°C
- 96-well processing plates with at least a 2 mL capacity
- 96-well microplate
- 2.0 mL microcentrifuge tubes
- 80% ethanol
- 100% ethanol
- 100% isopropanol
- Mineral oil
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)

Before starting:
- Prepare the PHM Buffer and RMP Buffer according to the instructions on the bottles
- Prepare 80% ethanol
- Set incubator to 90°C

Some of the buffers included in the Mag-Bind® FFPE DNA/RNA 96 Kit contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions to guanidine containing sample-preparation waste.

Please refer to the product manual for RNA only protocol.

Sequential Protocol

DNA Purification Procedure

LYSE

1. Transfer the FFPE samples to a 96-well processing plate (not provided) or 2.0 mL microcentrifuge tube (not provided). Add 300 µL mineral oil (not provided). Seal the plate with sealing film (not provided). Incubate for 3 minutes at 90°C. Remove the sealing film.
2. Add 400 µL FDR Buffer. Seal the plate with sealing film or cap the tube. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase. Incubate for 30 minutes at 90°C with constant shaking. Remove the sealing film or open the tube.
3. Add 20 µL Proteinase K Solution. Seal the plate with sealing film or close the tube. Briefly shake the plate for 30 seconds. Incubate for 30 minutes at room temperature.
4. Incubate for 1 hour at 90°C shaking at 300 rpm. Centrifuge at 2,000*g* for 1 minute. Remove the sealing film or open the tube. Transfer 325 µL of the lower aqueous layer to a new 96-well processing plate or microcentrifuge tube.

BIND

5. Add 650 µL MB4 Buffer and 10 µL Mag-Bind® Particles CH. Vortex for 10 minutes. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.
6. Aspirate the supernatant and **transfer to a new 96-well processing plate or 2.0 mL microcentrifuge tube.** Store the RNA-containing supernatant at room temperature or on ice up to 2 hours until DNA extraction is completed. If only DNA is desired, do not save the supernatant for RNA extraction. Remove the plate/tube containing the Mag-Bind® Particles CH from the magnetic separation device.

WASH

7. Add 400 µL RMP Buffer diluted with 100% isopropanol (see bottle for instructions). Vortex for 2 minutes.
8. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the plate/tube from the magnetic separation device.

WASH

9. Add 400 µL 80% ethanol. Vortex for 2 minutes.
10. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.
11. Remove the plate/tube from the magnetic separation device. Repeat Steps 9-10 for a second 80% ethanol wash step.
12. Leave the plate/tube on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles CH for an additional 10 minutes. Remove the plate/tube containing the Mag-Bind® Particles CH from the magnetic separation device.

ELUTE

13. Add 50-200 µL Elution Buffer. Vortex for 5 minutes to mix. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.
14. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate or 2.0 mL microcentrifuge tube. Store DNA at -20°C.

RNA Purification Procedure

BIND

1. Start with the supernatant (~845 µL) from Step 6 in DNA Purification Procedure. Add 845 µL 100% isopropanol and 10 µL Mag-Bind® Particles CH. Vortex or tip mix for 10 minutes.
2. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the plate/tube from the magnetic separation device.
3. Add 400 µL 80% ethanol. Vortex for 2 minutes. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.
4. Leave the plate/tube on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipette. Remove the plate from the magnetic separation device.

DNASE DIGESTION

5. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind® DNase I. Pipet up and down 20 times to mix. Let sit for 15 minutes at room temperature.
6. Add 225 µL PHM Buffer diluted with 100% ethanol (see bottle for instructions). Vortex for 5 minutes.
7. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the plate/tube from the magnetic separation device.

WASH

8. Add 400 µL 80% ethanol. Vortex for 2 minutes. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.
9. Remove the plate/tube from the magnetic separation device. Repeat Step 8 for a second 80% ethanol wash step
10. Leave the plate/tube on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles CH for an additional 10 minutes. Remove the plate from the magnetic separation device.

ELUTE

11. Add 50-200 µL Nuclease-free Water. Vortex for 5 minutes.
12. Place the plate/tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.
13. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate or 2.0 mL microcentrifuge tube. Store RNA at -80°C.