Lolina A/S

Address: Sindalsvej 30 8240 Risskov Danmark

Email: Info@lolina.dk

Website: https://lolina.dk

Product Specification

Product name	Lolina® Micro DNA Kit
Cat.No.	NaM102003
Size	50T/200T
Storage and shipping	Part I: Components are transported with ice packs and should be stored at -20 °C. Part II: Components are transported at room temperature and should be stored at room temperature. Product shelflife is 12 months.

Product Description

The Lolina® Micro DNA Kit utilizes Lolina® DNA Column M3 and a novel solution system, suitable for rapid extraction of genomic DNA from trace samples such as blood, forensic materials, dried blood spots, prescription pads, chewing gum, urine, and other trace samples. The extraction process does not require the use of toxic organic compounds such as phenol-chloroform or time-consuming ethanol precipitation. It is easy to operate and can complete the DNA extraction and purification of a single sample within 30 minutes. The Lolina® DNA Column M3 in the kit selectively adsorbs nucleic acids without adsorbing proteins, polysaccharides, and other non-nucleic acid substances, resulting in high DNA purity suitable for direct use in PCR, qPCR, and other experiments.

Components

Comp.	Comp. No.	Comp. Name	Size	
			50 T	200 T
Part I	NaM102003-A	Proteinase K (20 mg/mL)	1 mL	1 mL× 4
	NaM102003-B	Carrier RNA	200 µL	800 µL
Part II	NaM102003-C	Lolina® DNA Column M3	50 T	200 T
	NaM102003-D	2 mL Collection Tube M3	50 T	200 T
	NaM102003-E	LB Buffer M3	11 mL	44 mL
	NaM102003-F	BD Buffer M3	15 mL	60 mL

	NaM102003-G	PL Buffer M3	25 mL	100 mL
	NaM102003-H	Wash Buffer	13 mL	50 mL
	NaM102003-I	Elution Buffer	10 mL	20 mL

Transportation and Storage Methods

Precautions:

1. Avoid prolonged exposure of reagents to air to prevent evaporation, oxidation, and changes in pH. Always close the lids tightly after using each solution.

2. Precipitation may occur when the Binding Buffer (BD) and Protein Lysis Buffer (PL) are stored at low temperatures. If this happens, gently heat the solutions at 37°C until they become clear again. This does not affect their effectiveness.

3. The Binding Buffer (BD) and Protein Lysis Buffer (PL) contain irritating compounds. Wear latex gloves during operation to prevent skin contact. In case of skin or eye contact, rinse with plenty of water or saline solution immediately.

4. For your safety and health, wear a lab coat and disposable gloves while handling the reagents.

5. This product is intended for research purposes only.

Pre-experiment Preparation:

1. Prepare your own equipment and reagents: Benchtop centrifuge, shaker, water bath or metal bath, 100% ethanol, liquid nitrogen (for tissue grinding), 1.5 mL centrifuge tubes, etc.

2. All centrifugation steps should be performed at room temperature using a conventional benchtop centrifuge capable of reaching 12,000 rpm.

3. Before the first use, add four times the volume of anhydrous ethanol to the wash buffer (W*) bottle, mix thoroughly, and label it. If there is a significant discrepancy in volume due to improper transportation or storage, use a graduated cylinder to adjust the volume and then add the specified volume of anhydrous ethanol. After each use, tightly close the bottle cap to maintain the ethanol content.

4. Carrier RNA usage: If the starting material is limited, it is recommended to use Carrier RNA. If a large amount of nucleic acid yield is expected, users can choose whether to add Carrier RNA according to their needs. When using, add 4 μL of Carrier RNA storage solution to the Binding Buffer (BD) required for each sample extraction, and mix the Binding Buffer (BD) and Carrier RNA solution thoroughly by inversion (Binding Buffer (BD) tends to foam, so do not use vortex mixing). Alternatively, you can add the total amount of Carrier RNA needed to the total amount of Binding Buffer (BD) required and mix well for later use. The mixture is stable at room temperature for up to 24 hours.

Operating Procedure:

1) Pre-processing of Samples:

- Blood Sample

1. Pipette 10-100 µL ofblood into a 1.5 mL centrifuge tube. Add 10 µL ofProteinase K (20 mg/mL) and vortex thoroughly immediately to ensure thorough mixing.

   Note: Ifless than 100 µL ofblood is available, top up with lysis buffer LB.

2. Add 100 µL ofBinding Buffer (BD) and mix thoroughly. Incubate at 70 °C for 10 min.

   Note: If low DNA yield is expected, it is recommended to add 1 µL of Carrier RNA storage solution to 100 µL ofBinding Buffer (BD).

3. After cooling, add 50 µL of anhydrous ethanol (pre-cooled) and vortex immediately to ensure thorough mixing. Briefly centrifuge to collect droplets from the inside ofthe tube cap.

4. Allow to stand at room temperature (15-25 °C) for 3 min.

- Blood Card Sample

1. Use a hole puncher to collect 2-3 pieces of 3 mm diameter blood card paper into a 1.5 mL centrifuge tube. Add 180 µL of lysis buffer LB and 20 µL ofProteinase K (20 mg/mL), and vortex immediately to ensure thorough mixing.

2. Place the tube on a 56 °C orbital shaker at 900 rpm for 1 hour.

   Note: Alternatively, the tube can be placed in a 56°C water bath or metal bath for 1 hour, with vortexing for 10 seconds every 10 minutes during incubation.

3. Add 200 µL ofBinding Buffer (BD) and mix thoroughly.

4. Place the tube on a 70 °C orbital shaker at 900 rpm for 10 minutes.

   Note: Alternatively, the tube can be placed in a 70°C water bath or metal bath for 10 minutes, with vortexing for 10 seconds every 3 minutes during incubation.

- Microscopic Tissue

1. Collect a small amount oftissue (< 10 mg) into a 1.5 mL centrifuge tube. Add 180 µL of lysis buffer LB and 20 µL of Proteinase K (20 mg/mL), and vortex immediately to ensure thorough mixing.

2. Incubate the tube in a 56 °C water bath or metal bath for 1 hour until the solution becomes clear, gently shaking a few times during incubation to aid in lysis.

3. Add 200 µL ofBinding Buffer (BD) and mix thoroughly.

   Note: If low DNA yield is expected, it is recommended to add 2 µL of Carrier RNA storage solution to 200 µL ofBinding Buffer (BD).

4. After cooling, add 200 µL of anhydrous ethanol (pre-cooled) and vortex immediately to ensure

thorough mixing. Briefly centrifuge to collect droplets from the inside of the tube cap.

5. Allow to stand at room temperature (15-25 °C) for 5 minutes.

- Chewing Gum

1. Collect 30 mg of chewing gum into a 1.5 mL centrifuge tube. Add 280 µL of lysis buffer LB and 20 µL of Proteinase K (20 mg/mL), and vortex immediately to ensure thorough mixing.

2. Place the tube on a 56 °C orbital shaker at 900 rpm for 3 hours.

   Note: Alternatively, the tube can be placed in a 56°C water bath or metal bath for 3 hours, with vortexing for 10 seconds every 10 minutes during incubation.

3. Add 200 µL of Binding Buffer (BD) and mix thoroughly.

   Note: If low DNA yield is expected, it is recommended to add 2 µL of Carrier RNA storage solution to 200 µL of Binding Buffer (BD).

4. Place the tube on a 70 °C orbital shaker at 900 rpm for 1 hour.

   Note: Alternatively, the tube can be placed in a 70°C water bath or metal bath for 1 hour, with vortexing for 10 seconds every 10 minutes during incubation.

5. After cooling, add 200 µL of anhydrous ethanol (pre-cooled) and vortex immediately to ensure thorough mixing. Briefly centrifuge to collect droplets from the inside of the tube cap.

6. Centrifuge at 12,000 rpm for 5 minutes and collect the supernatant.

- Forensic Samples

1. Cigarette Butt:

   Take a 1 cm^2 piece of cigarette butt and cut it into 6 pieces. Transfer them to a 1.5 mL centrifuge tube. Add 300 µL of lysis buffer LB and 20 µL of Proteinase K (20 mg/mL), and vortex immediately to ensure thorough mixing.

   Hair:

   Cut 0.5-1 cm of hair close to the hair follicle and transfer it to a 1.5 mL centrifuge tube. Add 280 µL of lysis buffer LB, 20 µL of Proteinase K (20 mg/mL), and 20 µL of 1 M DTT solution (self-prepared), and vortex immediately to ensure thorough mixing.

2. Place the tubes on a 56 °C orbital shaker at 900 rpm for 1 hour.

   Note: Alternatively, the tubes can be placed in a 56°C water bath or metal bath for 1 hour, with vortexing for 10 seconds every 10 minutes during incubation.

3. Add 200 µL of Binding Buffer (BD) and mix thoroughly.

   Note: If low DNA yield is expected, it is recommended to add 2 µL of Carrier RNA storage solution to 200 µL of Binding Buffer (BD).

4. Place the tubes on a 70 °C orbital shaker at 900 rpm for 1 hour.

Note: Alternatively, the tubes can be placed in a 70°C water bath or metal bath for 1 hour, with vortexing for 10 seconds every 10 minutes during incubation.

5. Centrifuge at 12,000 rpm for 5 minutes and collect the supernatant.

2) DNA Extraction:

1. Insert the DNA binding column M3 into a 2 mL collection tube and set aside.

2. Add the pre-processed sample solution into the DNA binding column M3. Centrifuge at 12,000 rpm for 1 minute and discard the flow-through.

Note: The maximum volume of the mixed solution added each time should not exceed 650 µL. Multiple centrifugation steps may be necessary.

3. Add 500 µL of Proteinase K buffer (PL) to the column. Centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

4. Add 600 µL of wash buffer to the column. Centrifuge at 12,000 rpm at room temperature for 30 seconds and discard the flow-through.

Note: Ensure that the wash buffer contains anhydrous ethanol.

5. Repeat step 4 once.

6. Place the DNA binding column M3 back into the collection tube and centrifuge at 12,000 rpm at room temperature for 2 minutes to remove any residual wash buffer.

7. Place the DNA binding column M3 into a new 1.5 mL centrifuge tube. Add 20-50 µL of elution buffer to the center of the column. Let it stand at room temperature for 2 minutes. Then centrifuge at 12,000 rpm for 1 minute. Collect the filtrate, which is the DNA solution.

Note: To increase the yield, the elution buffer can be preheated to 70°C. Additionally, for higher yields, the DNA filtrate can be applied to the column again, left at room temperature for 2 minutes, and then eluted.

8. The DNA solution can be stored long-term at -20 °C.