MARINABIOLAB™ TOTAL NUCLEIC ACID ISOLATION KIT

RUO

REF: MBLM03 (100 Rxn)

Instruction for use

Product Name: MarinaBioLab™ TOTAL NUCLEIC ACID ISOLATION KIT
REF No: MBLM03
KIT COMPONENTS

	Component	Quantity	Storage
1	Binding Solution	53 mL	15°C to 25°C
2	Wash Buffer I	100 mL	
3	Wash Buffer II	100 mL	
4	Wash Buffer III	50 mL	
5	Elution Buffer	20 mL	
6	Proteinase K	1 mL	
7	Magnetic Beads	2 mL	
8	IFU	1	

INTENDED USE

MarinaBioLab™ Total Nucleic Acid İsolation Kit designed for the rapid isolation of nucleic acids (DNA, RNA) from virus, bacteria, and yeast, fungi in biofluid and transport media samples. You can use the nucleic acid purified with this kit in a broad range of molecular biology downstream applications, such as sequencing and qPCR.

PRODUCT USE LIMITS

- All chemicals in the kit are intended only for research purposes.
- Designed for professional use by well-trained personnel.
- Expired kits should not be used.
- Reliable results depend on storage conditions, transportation and correct sample collection methods.

MATERIALS AND EQUIPMENT TO BE SUPPLIED BY USER

ITEM	SOURCE
Instrument	
Magnetic particle processor (one of the following, depending on quantity/volume of sample to be processed):	
For standard volume sample [1]: KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
For large volume sample [2]: KingFisher™ Flex Magnetic Particle Processor with 24 Deep-Well Head	5400640
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Consumables	
Deep-well plates:	
For standard volume sample [1]: KingFisher™ deep-well 96 plate	95040450
For large volume sample [2]: KingFisher™ Flex 24 deepwell plate	95040470
96-well standard plates (for use with KingFisher™ Flex only; tip comb placement and eluate storage):	
KingFisher™ 96 KF plate	97002540

Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ Duo Prime 12-tip comb, for use with KingFisher™ deep-well 96 plate	97003500
KingFisher™ Duo Prime 6-tip comb, for use with KingFisher™ Flex 24 deep-well plate	97003510
KingFisher™ 96 tip comb for deep-well magnets, KingFisher™ Flex protocol only	97002534
KingFisher™ Flex 24 deep-well tip comb and plate, KingFisher™ Flex protocol only	97002610
Elution strip (for use with KingFisher™ Duo Prime only; elution step):	
KingFisher Duo elution strip	97003520
KingFisher Duo cap for elution strip	97003540
Equipment	
Adjustable micropipettors	
Multi-channel micropipettors	
Materials	
MicroAmp™ Clear Adhesive Film	
Conical Tubes (15 mL)	
Conical Tubes (50 mL)	
Reagent reservoirs	
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	
Reagents	
Ethanol, 100% (molecular biology grade)	
Nuclease-free Water	
PBS (1X), pH 7.4	

1] Standard volume sample is 200–400 µL.
2] Large volume sample is 500 µL–2 mL.

GENERAL GUIDELINES

• Perform all steps at room temperature (20–25°C), unless otherwise noted.
• Precipitates can occur if the Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the Binding Solution at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.
• Reagent Mix tables are sufficient for a single reaction. To calculate volumes for other sample numbers, see the per-well volume and add at least 10% overage.
• Vortex Binding Beads thoroughly before each use.
• Ensure that the beads stay fully mixed within the solution during pipetting.
• Avoid creating bubbles during mixing and aliquoting.
• Binding/Bead Mix is very viscous so pipet with care to ensure that the correct volume is added to the sample.

BEFORE USE OF THE KIT WITH STANDARD VOLUME OF VAGINAL MICROBIOTA AND URINARY TRACT SAMPLES (OPTIONAL)

1. Add 1 mL of sample per well of a 96 deep-well plate.
2. Cover the plate, then centrifuge for 15 minutes at 2,250 × g.

3. Remove as much of the supernatant as possible, then add 200 µL of 1X PBS to each sample.
4. Proceed to the nucleic acid purification according to the following protocols.

- "Perform total nucleic acid purification using KingFisher™ Flex (standard volume: 200–400 µL) " on page 2
- "Perform total nucleic acid purification using KingFisher™ Duo Prime (standard volume: 200–400 µL) "on page 6

TOTAL NUCLEIC ACID PURIFICATION USING KingFisher™ FLEX (Standard Volume: 200-400 µL)

Set Up the Instrument

Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

COMPONENT	TYPE
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block
IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.	

Ensure that the proper program (MVP_Ultra_Flex) has been downloaded from the product page and loaded onto the instrument.

Set Up the Processing Plate

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

PLATE ID	PLATE POSITION	PLATE TYPE	REAGENT	VOLUME PER WELL
Wash 1 Plate	2	Deep-well	Wash 1 Buffer	1,000 µL
Wash 2 Plate	3	Deep-well	Wash 2 Buffer	1,000 µL
Wash 3 Plate	4	Deep-well	Wash 3 Buffer	500 µL
Elution Plate	5	Deep-well	Elution Solution	60-100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Preparing Binding Bead Mix

- Vortex Beads vigorously to ensure they are homogenous.
- Prepare Binding Bead Mix according to the following table and sample input volume

COMPONENT	VOLUME PER WELL(1)
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume	**550 µL**
(1) Use 10% Overage calculation when making a master mix for use with multiple samples	

Mix well by inversion, then store at room temperature.

Digest with Proteinase K and Get Nucleic Acid Elution

When prompted (~20 minutes after start of protocol), remove Sample Plate from instrument.

- Add 10 µL of Proteinase K to each sample in the Sample Plate.
- Invert Binding Bead Mix gently to mix, then add 550 µL to each sample in the Sample Plate.
  - **Note:** Remix the Binding Bead Mix by inversion frequently during pipetting ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.
  - **DO NOT** use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.
- Load the Sample Plate back onto the instrument, then press Start.
- After the protocol is complete (~30 minutes after adding Binding Bead Mix), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

MBL Fast Extraction Method

TOTAL NUCLEIC ACID PURIFICATION USING KingFisher™ FLEX (Standard Volume: 200-400 µL)

Set Up the Instrument

Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

COMPONENT	TYPE
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block
IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.	

Ensure that the proper program (**MarinaBioLab_Fast**) has been downloaded from the product page and loaded onto the instrument.

Set Up the Processing Plate

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

PLATE ID	PLATE POSITION	PLATE TYPE	REAGENT	VOLUME PER WELL
Wash 1 Plate	2	Deep-well	Wash 1 Buffer	1,000 µL
Wash 2 Plate	3	Deep-well	Wash 2 Buffer	1,000 µL
Wash 3 Plate	4	Deep-well	Wash 3 Buffer	500 µL
Elution Plate	5	Deep-well	Elution Solution	60-100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Preparing Binding Bead Mix

- Vortex Beads vigorously to ensure they are homogenous.
- Prepare Binding Bead Mix according to the following table and sample input volume

COMPONENT	VOLUME PER WELL(1)
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume	**550 µL**
(1) Use 10% Overage calculation when making a master mix for use with multiple samples	

- Mix well by inversion, then store at room temperature.
- Prepare the Sample plate:
  - Add 10 µL of Proteinase K to each sample in the Sample Plate.
  - Invert Binding Bead Mix gently to mix, then add 550 µL to each sample in the Sample Plate.
    - **Note:** Remix the Binding Bead Mix by inversion frequently during pipetting ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.
    - **DO NOT** use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.
- When the sample plate is ready (*Each 200 – 400 uL sample should contain 10 uL of Proteinase K and 550 uL of the binding bead mixture*.) load it onto the instrument and begin the protocol
- After the protocol is complete (~30 minutes) immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage. The purified nucleic acid is ready for immediate use
- Alternatively, store the plate at –20°C for long-term storage.

TOTAL NUCLEIC ACID PURIFICATION USING KingFisher™ FLEX (Large Volume: 500 µL to 2 mL)

Set Up the Instrument

• Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

COMPONENT	TYPE
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block
IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.	

• Ensure that the proper program (MVP_Ultra_Flex) has been downloaded from the product page and loaded onto the instrument.

Set Up the Processing Plate

• Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

PLATE ID	PLATE POSITION	PLATE TYPE	REAGENT	VOLUME PER WELL
500 µL sample input				
Wash 1 Plate	2	24 Deep-well	Wash 1 Buffer	2,000 µL
Wash 2 Plate	3	24 Deep-well	Wash 2 Buffer	2,000 µL
Wash 3 Plate	4	24 Deep-well	Wash 3 Buffer	2,000 µL
Elution Plate	5	24 Deep-well	Elution Solution	150 µL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		
>500 µL - 1 mL sample input				
Wash 1 Plate	2	24 Deep-well	Wash 1 Buffer	4,000 µL
Wash 2 Plate	3	24 Deep-well	Wash 2 Buffer	4,000 µL
Wash 3 Plate	4	24 Deep-well	Wash 3 Buffer	2,000 µL
Elution Plate	5	24 Deep-well	Elution Solution	200 µL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		
>1 mL - 2 mL sample input				
Wash 1 Plate	2	24 Deep-well	Wash 1 Buffer	4,000 µL
Wash 2 Plate	3	24 Deep-well	Wash 2 Buffer	4,000 µL
Wash 3 Plate	4	24 Deep-well	Wash 3 Buffer	2,000 µL
Elution Plate	5	24 Deep-well	Elution Solution	250 µL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		

FOR SAMPLE IMPUT VOLUME	VOLUME PER WELL(1)
500 µL sample input	
Binding Solution	2,700 µL
Total Nucleic Acid Magnetic Beads	50 µL
Total volume	**2750 µL**
500 µL - 2 mL sample input	
Binding Solution	2,700 µL
Total Nucleic Acid Magnetic Beads	100 µL
Total volume	**2800 µL**
(1) Use 10% Overage calculation when making a master mix for use with multiple samples	

• Mix well by inversion, then store at room temperature

Digest with Proteinase K and Get Nucleic Acid Elution

• When instructed by the instrument (~20 minutes after the start of the protocol), remove the Sample Plate, then add Proteinase K to each sample according to the following table:

FOR SAMPLE IMPUT VOLUME	ADD PROTEINASE K
500 µL	25 µL
>500 µL – 2 mL	50 µL

• Invert the Binding Bead Mix gently to mix, then add Binding Bead Mix to each sample accord- ing to the following table

FOR SAMPLE IMPUT VOLUME	ADD BINDING BEAD MIX
500 µL	2,750 µL
>500 µL – 2 mL	2,800 µL

Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.
DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

• Immediately place the plate back onto the instrument, then follow the prompts on the instrument to allow the sample processing to proceed.
• At the end of the run (~45 minutes after adding the Binding Bead Mix), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.
The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

TOTAL NUCLEIC ACID PURIFICATION USING KingFisher™ DUO PRIME (Standard Volume: 200-400 µL)

Set Up the Instrument
• Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

COMPONENT	TYPE
Magnetic head	12-tip magnetic head
Heat block	12 well heat strip
IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.	

• Ensure that the proper program (MVP_Ultra_Duo) has been downloaded from the product page and loaded onto the instrument.

Set Up the Sample Plate and Elution Strip
Set up the Sample Plate and Elution Strip according to the following tables, respectively.

SAPLE PLATE			
ROW ID	**PLATE ROW**	**REAGENT**	**VOLUME PER WELL**
Sample	A	Sample	Varies
—	B	Empty	
Wash 1	C	Wash 1 Buffer	1,000 µL
—	D	Empty	
Wash 2	E	Wash 2 Buffer	1,000 µL
—	F	Empty	
Wash 3	G	Wash 3 Buffer	500 µL
Tip Comb	H	Tip Comb	

ELUTION STRIP			
ROW ID	**PLATE ROW**	**REAGENT**	**VOLUME PER WELL**
Elution	A	Elution Solution	60-100 µL

Preparing Binding Bead Mix

- Vortex Beads vigorously to ensure they are homogenous.
- Prepare Binding Bead Mix according to the following table and sample input volume:

COMPONENT	VOLUME PER WELL(1)
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume	**550 µL**
(1) Use 10% Overage calculation when making a master mix for use with multiple samples	

- Mix well by inversion, then store at room temperature.

Digest with Proteinase K and Get Nucleic Acid Elution

- When prompted (~20 minutes after start of protocol), remove Sample Plate from instrument.
- Invert Binding Bead Mix gently to mix, then add 550 µL to each sample in Row A of the Sample Plate

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

- Load the Sample Plate back onto the instrument, then press Start.
- After the protocol is complete (~30 minutes after adding Binding Bead Mix), immediately remove the Elution strip from the instrument. Cover with the Elution Strip Cap for temporary storage, or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

TOTAL NUCLEIC ACID PURIFICATION USING KingFisher™ DUO PRIME (Large Volume: 500 µL to 2 mL)

Set Up the Instrument

• Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

COMPONENT	TYPE
Magnetic head	6-tip magnetic head
Heat block	Both 6 well heat strips
IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.	

• Ensure that the proper program (MVP_Ultra_Duo_LV) has been downloaded from the product page and loaded onto the instrument.

Set Up the Instrument

24 Deep-Well Plate Layout (500 µL Sample Input)			
ROW ID	**PLATE ROW**	**REAGENT**	**VOLUME PER WELL**
SAPLE PLATE			
Sample	A	Sample	Varies
Wash 1	B	Wash 1 Buffer	2,000 µL
Wash 2	C	Wash 2 Buffer	2,000 µL
Wash 3	D	Wash 3 Buffer	2,000 µL
ELUTION PLATE			
Elution	A	Elution Solution	150 µL
Tip Comb	B	6-Tip Comb in a 24 Deep-well plate	

24 Deep-Well Plate Layout (>500 µL - 1 mL Sample Input)			
ROW ID	**PLATE ROW**	**REAGENT**	**VOLUME PER WELL**
SAPLE PLATE			
Sample	A	Sample	Varies
Wash 1	B	Wash 1 Buffer	4,000 µL
Wash 2	C	Wash 2 Buffer	4,000 µL
Wash 3	D	Wash 3 Buffer	2,000 µL
ELUTION PLATE			
Elution	A	Elution Solution	200 µL
Tip Comb	B	6-Tip Comb in a 24 Deep-well plate	

24 Deep-Well Plate Layout (>1 mL - 2 mL Sample Input)			
ROW ID	**PLATE ROW**	**REAGENT**	**VOLUME PER WELL**
SAPLE PLATE			
Sample	A	Sample	Varies
Wash 1	B	Wash 1 Buffer	4,000 µL
Wash 2	C	Wash 2 Buffer	4,000 µL
Wash 3	D	Wash 3 Buffer	2,000 µL
ELUTION PLATE			
Elution	A	Elution Solution	250 µL
Tip Comb	B	6-Tip Comb in a 24 Deep-well plate	

Preparing Binding Bead Mix

- Vortex Beads vigorously to ensure they are homogenous.
- Prepare Binding Bead Mix according to the following table and sample input volume:

COMPONENT	VOLUME PER WELL(1)
500 µL sample input	
Binding Solution	2700 µL
Total Nucleic Acid Magnetic Beads	50 µL
Total volume	**2750 µL**
500 µL - 2 mL sample input	
Binding Solution	2700 µL
Total Nucleic Acid Magnetic Beads	100 µL
Total volume	**2800 µL**
(1) Use 10% Overage calculation when making a master mix for use with multiple samples	

- Mix well by inversion, then store at room temperature.

Digest with Proteinase K and Get Nucleic Acid Elution

- When instructed by the instrument (~20 minutes after the start of the protocol), remove the Sample Plate, then add Proteinase K to each sample according to the following table:

FOR SAMPLE IMPUT VOLUME	ADD BINDING BEAD MIX
500 µL	2750 µL
>500 µL - 2 mL	2800 µL

• Invert the Binding Bead Mix gently to mix, then add Binding Bead Mix to each sample in Row A of the Sample Plate according to the following table:

FOR SAMPLE IMPUT VOLUME	ADD BINDING BEAD MIX
500 µL	2750 µL
>500 µL - 2 mL	2800 µL

Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

• Immediately place the plate back onto the instrument, then follow the prompts on the instrument to allow the sample processing to proceed.

• At the end of the run (~45 minutes after adding the Binding Bead Mix), immediately remove the Elution Plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

SYMBOL USE

Symbol	Meaning
	Manufacturer
	Use- by Date (Expiration Date) YYYY-MM
COMPONENT -	Negative Control
COMPONENT +	Positive Control
	Temperature Limit (Store temperature)
	Keep away from light
	Protect from heat and radioactive sources
	Do not use if package is damaged and consult instruction for use
IVD	For In Vitro Diagnostic Use
LOT	Batch code
REF	Catolog number
	Non-sterile
	Consult instruction for use
	Caution
	Keep away from water/ moisture
	Fragile, handle with care
	Keep it upright

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