Water DNA/RNA Magnetic Bead Kit

English Version

Magnetic bead based nucleic acid extraction kit

IDEXX

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Name and Intended Use

The Water DNA/RNA Magnetic Bead Kit is designed for the isolation of DNA and RNA from wastewater concentrates.

General Information

The Water DNA/RNA Magnetic Bead Kit can be used to process individual samples in microcentrifuge tubes, or with optional automated magnetic separators, such as Kingfisher™ or MagMax™ purification systems for high throughput sample processing. Samples are initially treated with Binding Buffer (BB) and Proteinase K (PK) to release DNA/RNA and inactivate nucleases. Binding of nucleic acids to paramagnetic beads takes place in the presence of the Binding Buffer (BB). After magnetic separation, the beads are washed to remove inhibitors, proteins, and other contaminants using two Washes (W1 and W2). After a drying step, the purified RNA/DNA is eluted with a small volume of Elution Buffer (EB).

Materials and Storage (Safety Information is on page 7)

Component		Quantity (4 x 96)	Storage	
PK	**Proteinase K**	3 x 7 mL	At receipt 2–26°C	After first use –25 to –15°C
BB	**Binding Buffer**	120 mL	15–26°C	
W1	**Wash 1**	132 mL		
W2	**Wash 2**	146 mL		
EB	**Elution Buffer**	60 mL		
MB	**Magnetic Beads**	8 mL		

Materials Required but Not Provided

- Absolute Ethanol, ACS grade or equivalent
- 2-propanol, ACS grade or equivalent
- Nuclease-free microcentrifuge tubes
- Magnetic separator rack
- Heat block
- Vortex mixer
- Personal protective equipment (gloves, safety glasses, lab coat)
- Nuclease-free, aerosol-resistant pipette tips (wide bore tips may be necessary for some sample types)
- Pipettes (5-1000 μL)
- Seracare AccuPlex™ SARS-CoV-2 Verification Panel (Material Number: 0505-0129) or suitable alternative

For optional automated processing:

- Automated magnetic processor (such as Kingfisher™)
- Deep well plates for lysis, binding and wash steps (see Ordering Information section)
- Elution (U bottom) plate or strip for eluted samples (see Ordering Information section)
- Tip combs (for automated processors) (see Ordering Information section)

Laboratory Practices and Warnings

- Do not use reagents past expiration date.
- Wear powder-free gloves when working with the reagents and nucleic acids.
- To avoid cross-contamination, use nuclease-free, aerosol-resistant pipette tips for all pipetting, and physically separate the workplaces for nucleic acid extraction/handling, PCR setup and PCR.
- Solutions BB and W1 contain chaotropic salts. Wear appropriate personal protective equipment (gloves, safety glasses, lab coat etc.) when handling.
- See additional safety information at the end of this document.

General Considerations

Handling of Magnetic Beads

- Before distributing the beads, shake or vortex the bottle to ensure that the beads are completely resuspended.

Reagent Preparation

Note: The Binding Buffer and Wash 1 contain components that may precipitate in cool temperatures (2–15°C). Before starting a preparation, visually inspect these components. If salt precipitation is observed, warm the solution to 37°C to dissolve the precipitated salts.

Reconstitute Proteinase K (PK)

Add 7.0 mL Elution Buffer to each vial prior to use. Mix well and mark the label to indicate that diluent has been added to the vial. Store reconstituted PK solution in aliquots at –25 to –15°C.

Preparation of Binding Buffer and Wash solution

Refer to the table below to prepare the Binding Buffer and Wash solutions. Once alcohol has been added to the bottles, check the box on the outer label. The expiration is the same as that listed on the component label.

Component	Starting Volume	Alcohol Addition
Binding Buffer	120 mL	100 mL 2-propanol
Wash 1	132 mL	80 mL ethanol
Wash 2	146 mL	300 mL ethanol

All other components are provided ready-to-use and should be stored at 15–26°C until expiration.

Water DNA/RNA Magnetic Bead Quick Reference

Lysis/Binding

1

1. Working Solution calculation:

Reagent	Volume per Sample
Binding Buffer (BB)	500 µL
Proteinase K (PK)	50 µL
Magnetic Beads (MB)	20 µL

2. 200 µL sample
3. Mix, incubate 10 minutes at 58°C
4. Separate beads

Wash Magnetic Beads

2

1. 500 µL Wash 1; separate beads
2. 500 µL Wash 2; separate beads
3. 500 µL Wash 2; separate beads
4. Dry beads 5–10 minutes at 18–26°C

Elute Nucleic Acids

3

1. 100 µL Elution Buffer
2. Mix, incubate 10 minutes at 18–26°C
3. Separate beads
4. Transfer eluate to clean plate or tube

See detailed protocol on the following page.

Water DNA/RNA Magnetic Bead Protocol (Manual Microcentrifuge Tube Procedure)

Preparation of Lysis/Binding Working Solution and Sample Lysis

Calculate the amount of Working Solution required. Prepare an additional 10% to allow for pipetting loss.
Lysis Working Solution calculation:

Reagent	Volume per Sample
Binding Buffer (BB)	500 µL
Proteinase K (PK)	50 µL
Magnetic Beads (MB)	20 µL

1. Prepare Working Solution by mixing reagents in the order listed above. Mix beads to ensure homogenous solution prior to pipetting. The Working Solution can be stored at 18 to 26°C for up to 1 hour prior to use. Longer storage time may result in diminished lysis efficiency.
2. Mix Working Solution thoroughly with inversion before use to ensure that beads are in a homogenous solution.
3. Add 570 ±10 µL Working Solution to a microcentrifuge tube.
4. Prior to addition, mix the wastewater concentrate by pipetting up and down several times.
5. Add 200 ±5 µL of wastewater concentrate to the sample tube and mix well.
6. Incubate 10 minutes at 58 ±2°C in a dry heat block. Periodically vortex tube to keep magnetic beads suspended and break apart any aggregates that may form.
7. Flash spin tubes to collect all liquid into the bottom of the tube.

Wash Magnetic Beads

1. Separate the magnetic beads by placing the sample tube on the magnetic separator. Wait 1–2 minutes until all the beads have been attracted to the magnets. Remove supernatant with a pipette, taking care not to disturb the magnetic beads.
2. Remove the tube from the magnetic separator. Add 500 ±20 µL Wash 1 and mix well by pipetting up and down to completely resuspend the beads.
3. Repeat Step 1 to remove Wash 1.
4. Remove the tube from the magnetic separator. Add 500 ±20 µL Wash 2 and mix well by pipetting up and down to completely resuspend the beads.
5. Repeat Step 1 to remove Wash 2.
6. Repeat Step 4 to resuspend the beads in Wash 2 again.
7. Repeat Step 1 to remove Wash 2.
8. Dry the beads in the open tube for 5–10 minutes at 18–26°C.

Elute Nucleic Acids

1. Add 100 µL Elution Buffer to the sample tube and mix well by pipetting up and down to completely resuspend the beads.
2. Incubate 10 minutes at 18–26°C. Periodically mix tube to keep magnetic beads suspended.
3. Separate the magnetic beads by placing the tube on the magnetic separator. Wait 1–2 minutes until all the beads have been attracted to the magnets.
4. Transfer the supernatant containing purified nucleic acid to a new tube.
5. Store the purified nucleic acid at 2–8°C for use within 6 hours, or at –25 to –15°C for up to 1 month, or at –80°C for long-term storage.

Procedural Notes

- The amount of sample mixing required to keep beads suspended during the binding and elution steps will vary from sample to sample. Proper suspension of the beads should be verified by visual inspection.
- If necessary, flash spin tubes to collect liquid at the bottom after mixing.

Water DNA/RNA Magnetic Bead Protocol (Automated Procedure)

Instrument Run

Contact IDEXX Technical Service for assistance with obtaining and installing the correct method file for your instrument prior to performing extractions.

Preparation of Sample Lysis Plate

1. Calculate the amount of Working Solution required. Prepare an additional 10% to allow for pipetting loss. Lysis Working Solution calculation:

Reagent	Volume per Sample
Binding Buffer (BB)	500 µL
Proteinase K (PK)	50 µL
Magnetic Beads (MB)	20 µL

2. Prepare Working Solution by mixing reagents in the order listed above. Mix beads to ensure homogenous solution prior to pipetting.
3. Mix Working Solution thoroughly with inversion before use to ensure that beads are in a homogenous solution. Store at 18–26°C for up to 1 hour prior to use. Longer storage time may result in diminished lysis efficiency.
4. Add 570 µL (±10 µL) Working Solution to appropriate wells of a 96-well deep well plate.
5. Add 200 µL (±5 µL) of wastewater sample to the wells.

Preparation of Wash and Elution Plates

Wash/Elution plates (Kingfisher FLEX):

Wash Plate 1– Add 500 µL (±20 µL) Wash 1 to wells of a deep well plate.
Wash Plate 2– Add 500 µL (±20 µL) Wash 2 to wells of a deep well plate.
Wash Plate 3– Add 500 µL (±20 µL) Wash 2 to wells of a deep well plate.
Elution Plate– Add 100 µL Elution Buffer (EB) to wells of a standard (200 µL) 96 well plate.

Wash/Elution wells for Kingfisher DUO and DUO Prime:

Use the rows of a single deep well plate for samples and wash solutions, and a separate elution strip for Elution Buffer:

Row A: Add sample and working solution to wells of row A
Row B: Place the tip comb in row B
Row C–E: Rows C, D and E are not used– remain empty
Row F: Add 500 µL (±20 µL) Wash 1 to wells of row F
Row G: Add 500 µL (±20 µL) Wash 2 to wells of row G
Row H: Add 500 µL (±20 µL) Wash 2 to wells of row H
Elution Strip: Add 100 µL Elution Buffer (EB) to wells of an elution strip

Complete the Run

Run the appropriate Method file for the instrument and insert plates/strip as indicated on the instrument display.

1. The instrument stops after the final elution step.
2. Follow the instructions on the instrument's display and unload the plate or strip from the instrument. Cover plate or elution strip wells with foil sealer.
3. Store the purified nucleic acid at 2–8°C for use within 6 hours, at –25 to –15°C for up to 1 month, or at –80°C for long-term storage.

Quality Control procedure:

The following controls should be performed in parallel with each set of wastewater samples that are extracted.

- Extraction Positive Control (Accuplex SARS-CoV-2 Verification Panel):
  An extraction control containing SARS-CoV-2 RNA should be extracted and tested with each set of samples. The extraction positive control is used to demonstrate successful recovery of RNA during the extraction process and should test positive for the SARS-CoV-2 target during PCR. IDEXX recommends using the Accuplex Verification Panel (see ordering information), which contains a recombinant virus engineered to harbor SARS-CoV-2 RNA sequences; this provides a full extraction control that requires lysis and recovery of single-stranded RNA from an encapsulated viral particle to produce a positive result during PCR. To perform the extraction positive control, use 200 µL of the "Member 1" dilution provided in the Verification Panel for the sample during extraction.

  Alternatively, samples that have been confirmed positive for SARS-CoV-2 may be used for the extraction positive control. Such samples should be available in enough volume to be used across multiple runs, tested prior to use, and used at an appropriate dilution to ensure the expected positive results are obtained.

- Extraction Negative Control (PCR Grade Water):
  A "no template" (negative) control containing no nucleic acids should be extracted and tested with each set of wastewater samples to verify the absence of nucleic acid contamination in the extraction reagents and materials. To perform the extraction negative control, use 200 µL of PCR Grade Water as the sample during extraction. The Extraction Negative Control should give a negative result for the SARS-CoV-2 target.

Ordering Information (Automated Procedure)

Product	Vendor	REF
Poly (A)	Millipore Sigma	10108626001
Wide Bore tips	Thermo Fisher Scientific	2079G (1000 µL) 2069G (200 µL)
96 deep-well plate (FLEX and DUO)	Thermo Fisher Scientific	95040450
96-well microplate (FLEX)	Thermo Fisher Scientific	97002540
Tip comb (FLEX)	Thermo Fisher Scientific	97002534
Tip comb (DUO)	Thermo Fisher Scientific	97002070
Elution strip (DUO)	Thermo Fisher Scientific	97003520
Sealing Foil (50 pieces)	IDEXX	98-56152-00 (50 pieces)

Safety Information

The following components of the Water DNA/RNA Magnetic Bead Kit contain hazardous contents. Wear gloves and goggles and follow the safety instructions given in this section.

GHS Classification

Component	Hazardous Substances		GHS Symbol	Hazard phrases	Precaution phrases
Binding Buffer (BB)	Guanidine hydrochloride 35–50%		Warning	302, 315, 319	280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313
Wash 1 (W1)	Guanidine hydrochloride 35–50%		Warning	302, 315, 319	280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313
PK	Proteinase K (5–10%)		Danger	334	261, 285, 304+341, 342+311, 501

Hazard phrases

H 302 Harmful if swallowed.
H 315 Causes skin irritation.
H 319 Causes serious eye irritation.
H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precaution phrases

P 233 Keep container tightly closed.
P 261 Avoid breathing mist/vapors.
P 280 Wear protective gloves and eye protection.
P 285 In case of inadequate ventilation wear respiratory protection.
P 301+312 IF SWALLOWED: Call a POISON CENTER/ doctor/…/if you feel unwell.
P 302+352 IF ON SKIN: Wash with plenty of water/…
P 304+341 IF INHALED: If breathing is difficult, remove person to fresh air and keep comfortable for breathing.
P 305+351 +338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P 330 Rinse mouth.
P 332+313 IF skin irritation occurs: Get medical advice/attention.
P 337+313 IF eye irritation persists get medical advice/attention.
P 342+311 IF experiencing respiratory symptoms: Call a poison center/doctor.
P 501 Dispose of contents/container in accordance with local/regional/national/international regulations.

For further information, please see Material Safety Data Sheets.

For Technical Support, please call:
North / South America: 1 207 556 4496 / 1 800 321 0207
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06-0014721-00

Symbol Descriptions

	Batch Code (Lot)
	Catalog Number
	Use by date
	Manufacturer
	Temperature limitation

Manufactured in France for
IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092 USA
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