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 samples.

Overview of IDEXX materials and procedures validated for quantification of SARS-CoV-2 in wastewater

IDEXX has validated an end-to-end protocol for detecting SARS-CoV-2 in wastewater and offers materials and/or procedures for each required step in the process, as detailed below. IDEXX has validated that these protocols and materials reliably quantify SARS-CoV-2 in untreated wastewater. Validation data is available and demonstrates strong repeatability and sensitivity of the end-to-end protocol and materials. Please contact IDEXX Technical Support (contact information below) to request the validation report.

While IDEXX has validated the entire procedure, individual components of the procedure can be used independently. For example, the IDEXX Water DNA/RNA Magnetic Bead Kit and SARS-CoV-2 RT-PCR Test can be used with a different concentration method than the example PEG concentration. Similarly, the IDEXX Water SARS-CoV-2 RT-PCR Test can be used with different extraction methods, and so on.

Because each component of the test can be used independently, procedures for each component of the test are detailed in separate documents. To use the entire, end-to-end and validated procedure, it is recommended to reference the following documents in the order listed in the table below.

Test component	Description	Document
Concentration	Used to concentrate the viral particles and nucleic acids present in a wastewater sample to improve limit of detection	Sample Concentration Protocol for wastewater surveillance for SARS-CoV-2
Nucleic Acid Extraction	Used to extract and purify nucleic acids from the concentrated sample	This document
Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)	Used to quantify the amount of SARS-CoV-2 RNA in the extracted sample via amplification of specific genetic sequences	IDEXX Water SARS-CoV-2 RT-PCR Test Product Insert

In addition to the documentation detailed in the above table, a description of available and compatible controls is also available: Available and Compatible Controls for Quantifying SARS-CoV-2 in Wastewater with IDEXX test kits

General Information

The Water DNA/RNA Magnetic Bead Kit can be used to process individual samples in microcentrifuge tubesand may be adapted for use with optional automated magnetic separators 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).

The IDEXX Water Internal Control (IC) may be added to the lysis/binding solution if the purified nucleic acids will be analyzed subsequently with the IDEXX Water SARS-CoV-2 RT-PCR Test. The IC is not compatible with other RT-PCR tests for SARS-CoV-2. The IC provides an internal positive control to verify successful purification of nucleic acids from each sample and indicate the potential for PCR reaction inhibition. The IC reaction is multiplexed with SARS-CoV-2 and both targets are detected simultaneously in the same RT-PCR reaction on different fluorescent channels. More information about IC use and results interpretation is available in the product insert for the Water SARS-CoV-2 RT-PCR Test kit.

Optional Carrier RNA (Poly A) may be needed when extracting certain QC materials from non-wastewater matrices. Carrier RNA is not recommended for use with concentrated wastewater samples. Carrier RNA is not included in the kit and can be purchased separately (see Optional Materials below). For more information on performing validation experiments, please contact IDEXX Water Technical Support.

Materials and Storage (Safety Information is on page 7)

Component		Quantity (4 x 96)	Storage	
PK	**Proteinase K**	3 x 7 mL	At receipt	After reconstitution
			2–26°C	–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 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 consistent with current guidelines for handling infectious samples
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes (5-1000 μL)
- Seracare AccuPlex™ SARS-CoV-2 Verification Panel (Material Number: 0505-0168) or suitable alternative

Optional Materials

- IDEXX Water Internal Control (IDEXX part number: 99-57010-00)
- Carrier RNA (Poly A), (Millipore Sigma part number: 10108626001)

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/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

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. Ensure the correct alcohol is added to the correct component (2-propanol vs. ethanol). 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.

Ensure the correct alcohol is added to the correct component (2-propanol vs. ethanol). The Binding Buffer and Wash solutions cannot be used if not prepared correctly.

Reconstitute Optional Carrier RNA (Poly A)

Rehydrate lyophilized Carrier RNA and dilute to a working solution of 4 mg/mL. Store rehydrated Carrier RNA solution in aliquots at –25 to –15°C.

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
IDEXX Water Internal Control*	2 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 a clean tube

See detailed protocol on the following page.

***Note:** The IDEXX Water Internal Control is provided separately and is an optional component (see Optional Materials above). It is only used when purified nucleic acids are analyzed with the IDEXX Water SARS-CoV-2 RT-PCR Test.

Water DNA/RNA Magnetic Bead Protocol

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	500 µL
Proteinase K	50 µL
Magnetic Beads	20 µL
IDEXX Water Internal Control*	2 µL*
Total*	572 µL

***Note:** The IDEXX Water Internal Control is provided separately and is an optional component (see Optional Materials above). It is only recommended for use when purified nucleic acids are analyzed with the IDEXX Water SARS-CoV-2 RT-PCR Test. The total volume per sample will only be 570µL when the Internal Control is not used.

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 572±10 µL Working Solution to a microcentrifuge tube.
   Note: if the Internal Control is not used, 570±10µL should be added.
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.
   Note: 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.
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 and dispose of 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. It is recommended to leave the tubes in the magnetic separator during this step.

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.
   Note: 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.
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 Note

If necessary, flash spin tubes to collect liquid at the bottom after mixing.

Automated Protocol

The above procedure may be adapted for use with automated processing equipment for high-throughput extraction. Contact IDEXX Technical Support for details.

Quality Control Procedure:

The following controls should be included with each set of wastewater samples that are extracted.

- Extraction Positive Control:
  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 "Materials Not Provided" above), 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, it is recommended to use 200 µL of the "Member 1" dilution provided in the Verification Panel in place of the wastewater concentrate during extraction to provide the strongest signal. Please contact Technical Support for more information about use of other dilution levels.
- Extraction Negative Control:
  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.
- Optional Internal Control (IC):
  If samples are analyzed using the IDEXX Water SARS-CoV-2 RT-PCR Test and the optional Water Internal Control was added to the Lysis/Binding Working Solution prior to nucleic-acid purification as described above, then a multiplexed internal positive control is available for each sample to demonstrate successful purification of nucleic acids and indicate the potential for PCR inhibition. During RT-PCR, the Internal Control is detected on a different fluorescence channel from SARS-CoV-2. When the Internal Control is used, the Extraction Negative Control will continue to provide a negative result for SARS-CoV-2 and will also provide a positive result on the additional fluorescence channel that is used to interpret Internal Control results for each wastewater sample. Thus, the Extraction Negative Control should be included in the same extraction run as the wastewater samples to be analyzed with the Internal Control. Additional information on the Internal Control and interpretation of results is available in the product insert for the Water SARS-CoV-2 RT-PCR Test kit.

Note: Alternate materials may be used in place of the recommendations above. For more information, please refer to Available and Compatible Controls for Quantifying SARS-CoV-2 in Wastewater with IDEXX test kits or contact IDEXX Technical Support. If an alternate QC material is used for the Extraction Positive Control, it may be recommended to add Carrier RNA to the sample before extraction to ensure quantitative recovery. Purification of nucleic acids from other QC materials, for example a process control, may also require addition of Carrier RNA for best results. Please contact IDEXX Technical Support for details.

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:
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Symbol Descriptions

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

Manufactured in France for
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