OPTI* DNA/RNA Magnetic Bead Kit

English Version

Magnetic bead based nucleic acid extraction kit

IVD CE

For *in vitro* diagnostic use only

REF 99-58015

Version
06-58015-01

Approval Date: 07-OCT-2020

OPTIMedical

OPTI* DNA/RNA Magnetic Bead Kit

Name and Intended Use

The OPTI DNA/RNA Magnetic Bead Kit is designed for the isolation of DNA and RNA from respiratory samples.

General Information

The OPTI DNA/RNA Magnetic Bead Kit can be used with automated magnetic separators, such as Kingfisher™ or MagMax™ purification systems for high throughput sample processing, or with manual magnetic separator systems for smaller sample numbers.

Samples are initially treated with Binding Buffer (BB) and Proteinase K (PK) to release DNA/RNA and inactivate nucleases. Optional carrier RNA (poly A), can improve binding of low amounts of nucleic acids to the magnetic beads. Carrier RNA may interfere with some downstream applications, such as cDNA synthesis. Carrier RNA is not included in the kit and should be purchased separately. 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 6)

Component		99-58015 Quantity (4 x 96)	Storage	
PK	**Proteinase K**	3 x 7 mL	At receipt	After first use
			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 Required but Not Provided

- Absolute Ethanol, ACS grade or equivalent
- 2-propanol, ACS grade or equivalent
- Pipettes (5–1000 μL)
- Nuclease-free, aerosol-resistant pipette tips (wide bore tips may be necessary for some sample types)
- Vortex mixer
- Personal protective equipment (gloves, safety glasses, lab coat)
- Optional- Carrier RNA (for example 10–20 μg Poly (A) per sample lysis)

Automated Processing:

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

Manual Processing:

- Magnetic separator for 96-well plate or micro-centrifuge tubes
- Plate shaker/incubator (96-well plate method)
- Deep-well plates (96-well plate method)
- Heat block for micro-centrifuge tubes (Individual tube method)
- Nuclease-free micro-centrifuge tubes (individual tube method)
- Optional- Repeater pipette and 50–300 μL multi-channel pipette

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.
- Binding Buffer and Wash 1 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. Reconstituted PK solution may be freeze/thawed up to 3 times.

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.

OPTI 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 60°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 56°C (automated) or 18–26°C (manual)
3. Separate beads
4. Transfer eluate to clean plate or tube

See detailed protocols on the following pages.

Preparation of Working Solution

1. Calculate the amount of Working Solution required. Prepare approximately 10% extra 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. If using optional Carrier RNA, it should be added to the Working Soluton.
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.

OPTI DNA/RNA Magnetic Bead Protocol (Automated)

Prior to starting the instrument run, obtain and install the correct method file for your instrument. Contact IDEXX Technical Service for assistance.

Kingfisher FLEX

Use deep well plates for samples and wash solutions, and a standard (200 µL) plate for Elution Buffer:

Sample Plate- Add 570 µL (±10 µL) of prepared Working Solution and 200 µL (±5 µL) of sample material to wells of a 96-well deep well plate.

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.

Kingfisher DUO and DUO Prime:

Use the rows of a single deep well plate for samples and wash solutions; use a separate elution strip for Elution Buffer.

Row A: Add 570 µL (±10 µL) of prepared Working Solution and 200 µL (±5 µL) of sample material to each well of Row A
Row B: Place the tip comb in row B
Row C–E: Rows C, D and E are not used and remain empty
Row F: Add 500 µL (±20 µL) Wash 1 to each well
Row G: Add 500 µL (±20 µL) Wash 2 to each well
Row H: Add 500 µL (±20 µL) Wash 2 to each well
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.

OPTI DNA/RNA Magnetic Bead Protocol (Manual)

This protocol is designed for use with 96-well plate magnetic separators with static pins or microfuge tube racks with suitable magnetic separators.

Sample Lysis/Binding

1. Prepare Working Solution as described in above.
2. Add 570 µL (±5 µL) Working Solution to appropriate wells of a 96-well deep well plate or tubes.
3. Add 200 µL (±5 µL) of sample material to the wells or tubes.
4. Mix well (pipette up and down multiple times, or vortex) to ensure that beads are in solution.
5. Incubate 12 (±2) minutes at 58ºC (±2ºC) with continuous shaking at 1200 (±100) rpm (plate) or periodic vortexing (tubes) to keep beads suspended in solution.

Wash Beads

1. Separate the magnetic beads by placing the plate or tubes on the magnetic separator. Wait 1–2 minutes until all the beads have been attracted to the magnets. Remove supernatant by pipetting or aspiration, taking care not to disturb the magnetic beads.
2. Remove the plate or tubes from the magnetic separator. Add 500 µL (±20 µL) Wash 1 to appropriate wells or tubes, and mix or shake (1–3 minutes) at 18–26°C until the beads are resuspended completely.
3. Repeat Step 1 to remove Wash 1.
4. Remove the plate or tubes from the magnetic separator. Add 500 µL (±20 µL) Wash 2 to appropriate wells or tubes, and mix or shake (1–3 minutes) at 18–26°C until the beads are resuspended completely.
5. Repeat Step 1 to remove Wash 2.
6. Remove the plate or tubes from the magnetic separator. Add 500 µL (±20 µL) Wash 2 to appropriate wells or tubes, and mix or shake (1–3 minutes) until the beads are resuspended completely.
7. Repeat Step 1 to remove Wash 2.
8. Dry the beads in open tubes or wells for 5–10 minutes at 18–26°C.

Elution of RNA/DNA from the Beads

1. Add 100 µL Elution Buffer to appropriate wells or tubes, Incubate 5–10 minutes at 18–26°C with continuous shaking at 1200 (±100) rpm (plate) or periodic vortexing (tubes) to keep beads suspended in solution.
2. Separate the magnetic beads by placing the plate or tubes on the magnetic separator. Wait 1–2 minutes until all the beads have been attracted to the magnets.
3. Transfer the supernatant containing purified nucleic acid to a fresh plate or tubes for immediate use or storage. *If a plate was used, cover with foil sealer (see ordering information)*.
4. 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.

Ordering information

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) Deep-well plate (50 pieces)	Thermo Fisher Scientific IDEXX	95040450 98-0014516-01 (50 pieces)
96-well microplate (FLEX) Microplate (48 pieces)	Thermo Fisher Scientific IDEXX	97002540 98-0014517-01 (48 pieces)
Tip comb (FLEX) Tip comb (FLEX) (100 pieces)	Thermo Fisher Scientific IDEXX	97002534 98-0014515-01 (100 pieces)
Tip comb (DUO)	Thermo Fisher Scientific	97002070
Elution strip (DUO)	Thermo Fisher Scientific	97003520
Sealing Foil (50 pieces)	IDEXX	98-56152-00 (50 pieces)
96-well magnetic separator	IDEXX	98-0014227-00

Safety Information

The following components of the Opti 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	264, 270, 280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313, 362+364
Wash 1 (W1)	Guanidine hydrochloride 35–50%		Warning	302, 315, 319	264, 270, 280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313, 362+364
PK	Proteinase K (5–10%)		Danger	334	261, 284, 304+340, 342+311

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 261 Avoid breathing mist/vapors.
P 264 Wash thoroughly after handling.
P 270 Do not eat, drink or smoke when using this product.
P 280 Wear protective gloves/eye protection/face protection.
P 284 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+340 IF INHALED: 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 362+364 Take off contaminated clothing and wash it before reuse.

For further information, please see Material Safety Data Sheets.

For In Vitro Diagnostic Use Only

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Contact your IDEXX area manager or distributor or visit our website: www.optimedical.com

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Symbol Descriptions

LOT	Batch Code (Lot)
SN	Serial Number
REF	Catalog Number
EC REP	Authorized Representative in the European Community
IVD	In Vitro Diagnostic
	Use by date
	Date of manufacture
	Manufacturer
	Temperature limitation
	Consult instructions for use
	Major change in the user instructions
CE	CE marking - European conformity

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