PrepSEQ™ Nucleic Acid Extraction Kit

Total Nucleic Acid (DNA and RNA) Extraction

Catalog Numbers 4480466 and 4428176

Pub. No. MAN0019336 **Rev.** C.0

Product description

The PrepSEQ™ Nucleic Acid Extraction Kit is designed for preparation of high-quality total nucleic acid (NA) from tissue, liquid, and swab samples. Magnetic beads allow efficient DNA and RNA capture and sample washing.

This user guide describes the following methods:

Kit contents and storage

Contents	Cat. No. 4480466 (100 isolations)	Cat. No. 4428176 (300 isolations)	Storage[1]
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol)[2]	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate[3]	2 × 26 mL	6 × 26 mL	
Elution Buffer	25 mL	3 × 25 mL	
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	–25°C to –15°C

[1] Refer to the product label for the expiration date.
[2] Add ~35 mL of 100% isopropanol to the empty bottle before use.
[3] Add 74 mL of 95% ethanol before use.

Note: Kit components may ship separately depending on configuration and storage conditions.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Equipment for manual extraction only	
DynaMag™-2 Magnet	12321D
Benchtop microcentrifuge	Eppendorf 5415 D or equivalent
Benchtop shaker with heating system	Eppendorf Thermomixer™
(Optional but recommended) Plate centrifuge	MLS

Item	Source
Equipment, consumables, and reagents for automated extraction only	
KingFisher™ mL Food Protection Purification System	5400050C
Heat block or water bath	MLS
KingFisher™ mL Food Protection Purification System Tubes and Tips	15951
Total RNA Control (Human)	4307281
Equipment for manual and automated extraction	
Laboratory mixer, vortex or equivalent	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Consumables	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
Microcentrifuge tubes, PCR clean, 1.5-mL	MLS
Reagents	
Ethanol, 95%	MLS
Isopropanol, 100%	MLS

Isolate total nucleic acid from tissue samples

1 Treat the samples with proteinase K and perform cell lysis

a. Place up to 100 mg of solid (tissue) sample in a 1.5-mL microcentrifuge tube.

b. Add 300 µL of PK Buffer and 10 µL of Proteinase K.

c. Incbate for 60 minutes at 45℃ and 1000 rpm in the thermomixer.

d. Centrifuge for 2 minutes ar 10,000 x *g*, then transfer the supernatant to a new 1.5-mL centrifuge tube.

e. Add 200 µL of Lysis Buffer, then vortex for 15 seconds.

2 Bind the nucleic acid to the magnetic beads

Vortex the Magnetic Particles until complete resuspension (approximately 5 seconds).

a. Add 35 µL of Magnetic Particles to the sample.

b. Vortex for 10 seconds at low speed.

c. Add 350 µL of Binding Solution, then vortex for 5 seconds.

d. Incubate for 10 minutes at room temperature shaking continuously.

e. Vortex for 10 seconds at low speed, then place the tube in the DynaMag™-2 Magnet.

f. Let the tube rest in the DynaMag™-2 Magnet until complete separation occurs (approximately 1-2 minutes).

g. Carefully discard the liquid phase without disturbing the Magnetic Particles pellet.

3	Wash the nucleic acid	**a.** Add 300 µL of Wash Solution to the tube, then vortex at medium speed for 5 seconds, or until the pellet is completely resuspended. **b.** Place the tube in the DynaMag™-2 Magnet, then let it rest for 30 seconds. **c.** Carefully discard the liquid phase without disturbing the Magnetic Particles pellet. **d.** Repeat the two last steps two more times.
4	Elute the nucleic acid	**a.** Air-dry the Magnetic Particles in the DynaMag™-2 Magnet with the lid open for 5 minutes. **b.** Add 50 µL of Elution Buffer. **c.** Close the lid, then vortex the tube at medium speed for 5 seconds. **d.** Incubate the tube for 5 minutes at 45°C. **e.** Vortex the tube at medium speed for 2 seconds, then place the tube in the DynaMag™-2 Magnet. **f.** Let the tube rest in the DynaMag™-2 Magnet for at least 1 minute. **g.** Transfer the liquid phase containing the total NA to a new tube for storage.

Isolate total nucleic acid from liquid samples

1	Treat the samples with proteinase K and perform cell lysis	**a.** Place 250 µL of liquid sample in a 1.5-mL microcentrifuge tube. **b.** Add 50 µL of PK Buffer and 10 µL of Proteinase K, then vortex for 15 seconds. **c.** Incbate for 25 minutes at 45°C and 1000 rpm in the thermomixer. **d.** Centrifuge for 2 minutes ar 10,000 x *g*, then transfer the supernatant to a new 1.5-mL centrifuge tube. **e.** Add Lysis Buffer up to 500 µL of total volume, then vortex for 15 seconds.
2	Bind the nucleic acid to the magnetic beads	Vortex the Magnetic Particles until complete resuspension (approximately 5 seconds). **a.** Add 35 µL of Magnetic Particles to the sample. **b.** Vortex for 10 seconds at low speed. **c.** Add 350 µL of Binding Solution, then vortex for 5 seconds. **d.** Incubate for 10 minutes at room temperature shaking continuously. **e.** Vortex for 10 seconds at low speed, then place the tube in the DynaMag™-2 Magnet. **f.** Let the tube rest in the DynaMag™-2 Magnet until complete separation occurs (approximately 1-2 minutes). **g.** Carefully discard the liquid phase without disturbing the Magnetic Particles pellet.
3	Wash the nucleic acid	**a.** Add 300 µL of Wash Solution to the tube, then vortex at medium speed for 5 seconds, or until the pellet is completely resuspended. **b.** Place the tube in the DynaMag™-2 Magnet, then let it rest for 30 seconds. **c.** Carefully discard the liquid phase without disturbing the Magnetic Particles pellet. **d.** Repeat the two last steps two more times.

4	Elute the nucleic acid	**a.** Air-dry the Magnetic Particles in the DynaMag™-2 Magnet with the lid open for 5 minutes. **b.** Add 50 µL of Elution Buffer. **c.** Close the lid, then vortex the tube at medium speed for 5 seconds. **d.** Incubate the tube for 5 minutes at 45°C. **e.** Vortex the tube at medium speed for 2 seconds, then place the tube in the DynaMag™-2 Magnet. **f.** Let the tube rest in the DynaMag™-2 Magnet for at least 1 minute. **g.** Transfer the liquid phase containing the total NA to a new tube for storage.

Isolate total nucleic acid from swab samples (manual method)

1	Perform cell lysis	**a.** Place the swab sample in a 1.5-mL microcentrifuge tube. **b.** Add 650 µL of Lysis Buffer, then vortex for 15 seconds. **c.** Incbate for 25 minutes at 45°C and 1000 rpm in the thermomixer. **d.** Centrifuge for 2 minutes ar 10,000 x *g*, then transfer 500 µL of supernatant to a new 1.5-mL centrifuge tube.
2	Bind the nucleic acid to the magnetic beads	Vortex the Magnetic Particles until complete resuspension (approximately 5 seconds). **a.** Add 35 µL of Magnetic Particles to the sample. **b.** Vortex for 10 seconds at low speed. **c.** Add 350 µL of Binding Solution, then vortex for 5 seconds. **d.** Incubate for 10 minutes at room temperature shaking continuously. **e.** Vortex for 10 seconds at low speed, then place the tube in the DynaMag™-2 Magnet. **f.** Let the tube rest in the DynaMag™-2 Magnet until complete separation occurs (approximately 1-2 minutes). **g.** Carefully discard the liquid phase without disturbing the Magnetic Particles pellet.
3	Wash the nucleic acid	**a.** Add 300 µL of Wash Solution to the tube, then vortex at medium speed for 5 seconds, or until the pellet is completely resuspended. **b.** Place the tube in the DynaMag™-2 Magnet, then let it rest for 30 seconds. **c.** Carefully discard the liquid phase without disturbing the Magnetic Particles pellet. **d.** Repeat the two last steps two more times.
4	Elute the nucleic acid	**a.** Air-dry the Magnetic Particles in the DynaMag™-2 Magnet with the lid open for 5 minutes. **b.** Add 50 µL of Elution Buffer. **c.** Close the lid, then vortex the tube at medium speed for 5 seconds. **d.** Incubate the tube for 5 minutes at 45°C. **e.** Vortex the tube at medium speed for 2 seconds, then place the tube in the DynaMag™-2 Magnet.

4 Elute the nucleic acid *(continued)*

f. Let the tube rest in the DynaMag™-2 Magnet for at least 1 minute.

g. Transfer the liquid phase containing the total NA to a new tube for storage.

Isolate total nucleic acid from swab samples (automated method with the KingFisher™ mL Food Protection Purification System)

For more information about using the KingFisher™ mL Food Protection Purification System, see *Thermo Scientific™ KingFisher™ mL User Manual* (Pub. No. 1508260).

1 Before you begin

- Ensure that the **PSNA_mL_300ul** script has been downloaded from the product page and loaded onto the KingFisher™ mL Food Protection Purification System.
- Ensure that a water bath or heating block is heated to 83°C.
- Label the following consumables for each sample to be processed and the negative extraction control:
  - One tube strip
  - Two 1.5-mL microcentrifuge tubes (nuclease free)

  Note: Up to 14 samples and 1 negative extraction control can be processed at a time on the KingFisher™ mL Food Protection Purification System.

2 Set up processing tubes

Vortex the Magnetic Particles until complete resuspension (approximately 5 seconds).

a. For the number of required reactions, prepare the Binding Mix according to the following table:

Reagent	Volume per well[1]
PK Buffer	340 µL
Binding Solution	325 µL
Magnetic Particles	25 µL
Proteinase K	10 µL
Total volume per well	**700 µL**

[1] Include 10% overage when making for multiple reactions.

b. Invert the Binding Mix 5 times gently to mix, then add 700 µL to **Tube A** of each tube strip. Include tube strips for each sample and negative extraction control.

Note: Remix the Binding Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Mix to the samples, as the high viscosity will cause variations in the volumes added.

c. Add 300 µL of Wash Buffer to **Tube B** and 300 µL of Wash Buffer to **Tube C** of each tube strip.

d. Add 100 µL of Elution Buffer to **Tube D** of each tube strip.

e. Add 1 µL of Total RNA Control (Human) to **Tube A** of each tube strip.

f. Vortex the swab sample tubes for 30 seconds.

g. Add 300 µL of a sample to **Tube A** of the corresponding, pre-labeled tube strip. Repeat for the remaining samples and tube strips.

h. Add 300 µL of Nuclease-free Water (not DEPC-Treated) to **Tube A** of the Negative Extraction Control tube strip.

3 Process the samples on the instrument

a. Load the prepared tube strips into the tray, then place the tray in the KingFisher™ mL Food Protection Purification System.

b. Fully insert the tip combs into the tip comb slots.

c. Select the **PSNA_mL_300ul** script, then press **Start**.

d. When prompted by the instrument, remove the tube-strip tray from the instrument.

e. For each tube strip, transfer the elution buffer (100 µL) from **Tube D** into one of the corresponding pre-labeled microcentrifuge tubes.

f. Cap the microcentrifuge tubes, then incubate at 83°C for 4 minutes.

g. Transfer the elution buffer from each microcentrifuge tube back into **Tube D** of the corresponding tube strip.

h. Load the tube-strip tray into the instrument, then restart the run.

i. After the run is complete, immediately remove the tube-strip tray from the instrument.

j. For each tube strip, transfer the elution buffer (100 µL) from **Tube D** into the second pre-labeled microcentrifuge tube.

Place the microcentrifuge tubes on ice for immediate use in real-time PCR. The extracted samples can be stored at -70°C for long-term storage (up to one year).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
C.0	14 December 2020	Add information about the KingFisher™ mL Food Protection Purification System.
B.0	04 August 2020	Add additional kit size (Cat. No. 4480466).
A.0	27 April 2020	New document.