applied biosystems

PrepSEQ™ Sample Preparation Kits

USER GUIDE

for use with:

PrepSEQ™ Nucleic Acid Sample Preparation Kit

PrepSEQ™ Residual DNA Sample Preparation Kit

Catalog Numbers 4413686 and A50485

Publication Number 4469838

Revision H

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Revision history: Pub. No. 4469838

Revision	Date	Description
H	9 June 2021	Added the PrepSEQ™ Nucleic Acid Sample Preparation Kit (Cat. No. A50485) used for extracting nucleic acid (DNA or RNA) from common sample matrices.
G	2 April 2021	Added the resDNASEQ™ Quantitative Plasmid DNA - Kanamycin Resistance Gene Kit (Cat. No. A50337).

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Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The PrepSEQ™ Residual DNA Sample Preparation Kit uses chemical lysis and magnetic beads to extract DNA from diverse sample types, including samples that contain high protein and low DNA concentration.

- The PrepSEQ™ Residual DNA Sample Preparation Kit extracts residual genomic DNA from products that are produced in cell lines such as CHO, *E. coli*, HEK293, Human, MDCK, NS0, *Pichia*, Sf9 and Baculovirus, and Vero or residual plasmid DNA for Kanamycin resistance. For quantification of residual DNA, we recommend using the resDNASEQ™ Quantitative DNA Kits as described in the *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836).
- The PrepSEQ™ Nucleic Acid Sample Preparation Kit extracts nucleic acid (DNA or RNA) from common sample matrices in different steps of the bioproduction workflow. For detection/quantification of viral RNA or DNA, we recommend using the ViralSEQ™ Real - Time PCR Kits as described in the *ViralSEQ™ Real-Time PCR Kits User Guide* (Pub. No. 4445235).

To ensure accurate quantitative results, extract each sample in triplicate and perform a single PCR reaction for each extraction.

Contents and storage

The kits contain reagents sufficient for 100 extractions.

Table 1 PrepSEQ™ Residual DNA Sample Preparation Kit (Cat. No. 4413686)

Contents	Amount	Storage
Box 1, PrepSEQ™ Nucleic Acid Extraction Kit		
Lysis Buffer	2 × 50 mL	Room temperature
Binding Solution (Isopropanol), empty bottle	1	
Wash Buffer Concentrate	2 × 26 mL	
Elution Buffer	25 mL	
Proteinase K (PK) Buffer Can be used for existing validated manual protocols.	50 mL	

Table 1 PrepSEQ Residual DNA Sample Preparation Kit (Cat. No. 4413686) *(continued)*

Contents	Amount	Storage
Proteinase K (PK) Buffer II[1] Recommended for new manual protocols. Required for automated protocols.	50 mL	Room temperature
Box 2, PrepSEQ™ Nucleic Acid Extraction Kit		
Magnetic Particles	2 × 1.5 mL	Room temperature
Box 3, PrepSEQ™ Nucleic Acid Extraction Kit		
Proteinase K, 20 mg/mL	1.25 mL	–20°C or below
Yeast tRNA, 10 mg/mL	0.5 mL	
Glycogen, 5 mg/mL	2 × 1.0 mL	

[1] Also sold separately (Cat. No. 4415320).

Table 2 PrepSEQ™ Nucleic Acid Sample Preparation Kit (Cat. No. A50485)

Contents	Amount	Storage
Box 1, PrepSEQ™ Nucleic Acid Extraction Kit		
Lysis Buffer	2 × 50 mL	Room temperature
Binding Solution (Isopropanol), empty bottle	1	
Wash Buffer Concentrate	2 × 26 mL	
Elution Buffer	25 mL	
Proteinase K (PK) Buffer Can be used for existing validated manual protocols.	50 mL	
Proteinase K (PK) Buffer II[1] Recommended for new manual protocols. Required for automated protocols.	50 mL	
Box 2, PrepSEQ™ Nucleic Acid Extraction Kit		
Magnetic Particles	2 × 1.5 mL	Room temperature
Box 3, PrepSEQ™ Nucleic Acid Extraction Kit		
Proteinase K, 20 mg/mL	1.25 mL	–20°C or below
PrepSEQ™ Residual DNA Sample Preparation Kit		
Proteinase K, 20 mg/mL	1.25 mL	–20°C or below

Table 2 PrepSEQ Nucleic Acid Sample Preparation Kit (Cat. No. A50485) *(continued)*

Contents	Amount	Storage
Yeast tRNA, 10 mg/mL	0.5 mL	–20°C or below
Glycogen, 5 mg/mL	2 × 1.0 mL	

[1] Also sold separately (Cat. No. 4415320).

Required materials not supplied for manual protocols

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	
Pharma Magnetic Stand-96 or Magnetic stand, 16-position	A31543 12321D
Block heater for use with 2-mL tubes. Manual DNA/RNA extraction involves two incubations at different settings, so two heaters may be convenient.	Major laboratory supplier (MLS)
Benchtop microcentrifuge for 1.5-mL and 2-mL tubes	MLS
Vortex-Genie™ 2T Mixer	VWR™ 14216-188, VWR™ 14216-186
Vortex Adapter-60, for use with the Vortex-Genie™	AM10014
Consumables	
Disposable gloves	MLS
Aerosol-resistant micropipette tips	MLS
PIPETMAN™ Pipettors, P1000, P200, P20 and P10: • Positive-displacement • Air-displacement • Multichannel	MLS
Nonstick, RNase*Zap*™-free Microfuge Tubes, 1.5-mL (1 box; 250 tubes/box)	AM12450
Safe-Lock PCR clean microcentrifuge tubes, round-bottom, 2-mL	VWR™ 62111-754

Item	Source
Reagents	
Ethanol, 95% **IMPORTANT!** Do not use denatured ethanol. It contains components that are not compatible with the protocol.	MLS
Isopropanol, 100%	MLS
5 M NaCl and 1 N NaOH solutions	MLS
Hydrochloric acid (HCl)	MLS
1X PBS (pH 7.4; free of Mg and Ca)	MLS

Required materials not supplied for automated protocols

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.

Table 3 Pharma KingFisher™ Flex-96 Deep-Well Magnetic Particle Processor (Cat. No. A31508) and Pharma MagMAX™ Express-96 instrument[1] accessories

Item	Source
Pharma MagMAX™ Express-96 DW plate	A31540
Pharma MagMAX™ Express-96 Deep-Well Tip Combs	A31537
Pharma KingFisher™ Flex Magnetic Head for 96 Deep-Well Plate	A31542
Pharma MagMAX™ 96 PCR Well Magnetic Head	4472991
Pharma MagMAX™ Express-96 Standard Plates	A31541

[1] This instrument is no longer available for purchase

Table 4 Additional materials

Item	Source
Consumables	
Disposable gloves	MLS
Aerosol-resistant micropipette tips	MLS

Item	Source
PIPETMAN™ Pipettors, P1000, P200, P20 and P10: • Positive-displacement • Air-displacement • Multichannel	MLS
Nonstick, RNase*Zap*™-free Microfuge Tubes, 1.5-mL (1 box; 250 tubes/box)	AM12450
Reagents	
Ethanol, 95% **IMPORTANT!** Do not use denatured ethanol. It contains components that are not compatible with the protocol.	MLS
Isopropanol, 100%	MLS
5 M NaCl and 1 N NaOH solutions	MLS
Hydrochloric acid (HCl)	MLS
1X PBS (pH 7.4; free of Mg and Ca)	MLS

Workflow

Manual DNA/RNA extraction	Automated DNA/RNA extraction
Digest the test samples and controls (page 15)	Prepare the plates (page 18)
▼	▼
Bind the DNA/RNA (page 15)	Prepare the lysis plate (page 19)
▼	▼
Wash the DNA/RNA (page 16)	Process samples on the instrument (page 19)
▼	
Elute the DNA/RNA (page 17)	

2 Prepare the reagents and samples

Prepare the reagents: before first use of the kit

Magnetic beads

1. Set a block heater to 37°C.
2. Incubate the Magnetic Particle suspension at 37°C for a minimum of 10 minutes with intermittent vortexing at 900 rpm, or until the particles are completely suspended.

Binding Solution

1. Add 30 mL of 100% isopropanol to the Binding Solution bottle.
2. Label the bottle to indicate that it contains isopropanol, then store the bottle at ambient temperature.

Wash Buffer Concentrate

1. Add 74 mL of 95% ethanol to one bottle of PrepSEQ™ Wash Buffer Concentrate, then mix completely.
2. Label the bottle to indicate that it contains ethanol, then store the bottle at room temperature.

Prepare reagents: before each use of the kit

Proteinase K (PK) mix

- Use Proteinase K (PK) Buffer II for new manual protocols and automated protocols.

  Note: Proteinase K (PK) Buffer is also provided in the kit for use with existing manual protocols that have been internally validated with this buffer.

- Prepare a fresh mix before each use of the kit.
- Include a 10% overage to account for pipetting losses.

Component	Number of extractions				
	1	7	10	13	25
Proteinase K, 20 mg/mL	10 µL	70 µL	100 µL	130 µL	250 µL
Proteinase K (PK) Buffer II or Proteinase K (PK) Buffer	60 µL	420 µL	600 µL	780 µL	1,500 µL

Lysis solution

- Prepare a fresh mixture immediately before use or during Proteinase K incubation.
- Prepare 360 µL (amount required) of lysis solution mix per sample.

Reagent	Volume for ~20 extractions
Glycogen, 5 mg/mL	180 µL
Yeast tRNA, 10 mg/mL	4 µL
Lysis Buffer	7,600 µL
Total	**7,784 µL**

Guidelines for optimal yields

- Maintain a homogenous suspension of the magnetic beads to maximize the surface area to which the DNA/RNA can bind. The appearance of the mixture should be homogenous after mixing.
- After drying, the DNA/RNA remains bound to the magnetic beads. Do not allow the magnetic beads to over-dry because this reduces the elution efficiency; over-dried beads are not easily resuspended.
- During manual elution, vortex every 2 minutes to assist elution. This will result in better yield during recovery.

Note: Some test samples cause the beads to adhere very firmly to the tube wall, while others form loose pellets that detach during the vortex steps. All pellets should dissolve with vortexing during heated elution. If vortexing does not result in full resuspension, then wash the beads off the tube by pipetting.

Note: White or brown precipitate may form in the Magnetic Particles tube if it is stored at 2–8°C. The precipitate will dissolve when it is heated to 37°C for a minimum of 10 minutes with intermittent vortexing. Make sure the precipitate is completely dissolved before using the beads.

Sample preparation guidelines

Sample dilution (if necessary)

Test samples from the early purification process often contain levels of DNA/RNA that are above the highest point of the assay standard curve. You must dilute these samples (from 1:100 up to 1:10,000) before PrepSEQ™ sample preparation.

- Dilute test samples before DNA/RNA extraction with a solution of 1X PBS (free of Mg and Ca) or 50 mM Tris, pH 8.0, 0.5 M NaCl.

  Note: Diluting samples in water or TE reduces extraction efficiency.

- Use the sample dilution buffer as the negative extraction control instead of water.
- Alternatively, dilute extracted DNA/RNA with elution buffer before running the PCR reaction.

Triplicate extractions

Triplicate extractions are required for post-PCR analysis calculation of mean quantity, standard deviation, and coefficient of variation.

In addition to test samples, we recommend triplicate extractions for controls (for an explanation of controls , see “Extraction control guidelines” on page 13).

Perform a single PCR reaction for each extraction.

The table below illustrates the total number of extractions required based on the 1, 2, and 3 samples extracted in a batch.

Table 5 Total number of extractions per batch of test samples

Number of test samples		Total number of extractions for the batch
1	3 extractions required for each: • Test sample • Test sample extraction/recovery control (ERC) • Negative extraction control[1]	9
2		15
3		21

[1] Optional during routine testing.

(Plasmid samples only) Add Yeast tRNA

Plasmid samples require the use of **Yeast tRNA** as a carrier during the extraction process.

1. Dilute the **Yeast tRNA**.

Table 6 Diluted Yeast tRNA

Component	Volume
Yeast tRNA (10mg/mL)	5 µL
1X PBS (free of Mg and Ca)	245 µL
Total	**250 µL**

2. Add 5 µL **Diluted Yeast tRNA** to 370 µL of each test sample before extraction. This is sufficient for triplicate 100 µL extractions.

Extraction control guidelines

We recommend that you use the following extraction controls:

Type of control	Contains	Number to run	Used to
Negative (NEG)[1]	1X PBS (free of Mg and Ca)	1 per batch of extractions	Test for cross-contamination of DNA/RNA extraction reagents.
Extraction/recovery (ERC)	Positive control from the kit	3 per sample	• Evaluate the efficiency of DNA or RNA extraction, recovery, and quantification from test samples. • Verify reagent and system performance.

[1] Optional during routine testing.

For the Extraction/recovery (ERC) :

- Prepare the control standard dilutions as described in the *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836) or the *ViralSEQ™ Real-Time PCR Kits User Guide* (Pub. No. 4445235).
- Add a volume of positive control standard dilution to each test sample so that the total DNA/RNA amount is 2–10 times the amount of DNA/RNA measured in the test sample *without* the addition of the DNA/RNA control.

  For example:
  - The DNA amount measured in a test sample is ≤1 pg.
  - To prepare a 10 pg ERC for a PCR elution volume of 50 µL, spike samples with 16.7 µL of the 3 pg/µL positive control standard dilution (SD3) = 50 pg spike to yield 10 pg per PCR reaction.
- Prepare three separate extractions for each test sample, then add the ERC to each reaction. Do not prepare a large volume of ERC, then aliquot it into three reactions.

Note: To calculate the efficiency of DNA/RNA recovery and quantification from the test samples, subtract the amount of DNA/RNA measured in the sample *without* the addition of DNA/RNA control from the amount of DNA/RNA measured in the ERC sample.

Manual protocol for DNA/RNA extraction

Digest the test samples and controls

1. Set a block heater to 56°C. If available, set a second block heater to 70°C.
2. Label 2-mL Safe-Lock tubes:
   - 3 for each sample
   - 3 for each sample + ERC
   - 3 for NEG
3. Add 100 µL of sample, sample + ERC, or 1X PBS (free of Mg and Ca) into each tube.

   Note: Ensure that **Diluted Yeast tRNA** was added to each plasmid sample. See "(Plasmid samples only) Add Yeast tRNA" on page 13.
4. Add 10 µL of 5 M NaCl and 70 µLProteinase K/Proteinase K Buffer II mix.
5. Briefly vortex and centrifuge.
6. Incubate at 56°C for 30 minutes.

   If only one block heater is available, after this incubation step is complete, reset the block heater to 70°C for the elution step.

   Note: For samples with high protein concentration, extending the incubation time to 60 minutes can increase recovery.
7. Cool samples to room temperature.
8. Add 360 µL freshly made Lysis solution mix to each tube.

Bind the DNA/RNA

1. Vortex the Magnetic Particles to resuspend the particles.

   Note: The appearance of the mixture should be homogeneous.
2. Add 30 µL of the Magnetic Particles to each tube.

3. Add 400 µL Binding Solution to each tube.

4. Mix and vortex the tubes:
   a. Close the caps, **immediately** invert each tube twice to mix.

   b. Vortex the tubes in the vortex adaptor for 5 minutes at 900 rpm.

5. Briefly centrifuge the tubes for 15 seconds at top speed (>15,000 × *g*) to collect the Magnetic Particles at the bottom of the tubes.

6. Place the tubes in the magnetic stand with the pellet against the magnet, then let the tubes stand for 5 minutes or until the solution is clear.

7. Without disturbing the magnetic beads, remove the supernatant using a PIPETMAN™ pipette or by aspiration.

Wash the DNA/RNA

For aspiration of liquid supernatants and wash buffers during sample preparation, we recommend attaching the waste-collection bottle to the vacuum using tubing that can accommodate 200-µL pipette tips.

1. Remove the tube rack (with tubes) from the magnetic stand, then add 300 µL of Wash Solution to the tubes. Vortex the tubes for 5 seconds at room temperature at 900 rpm.

2. Centrifuge the tubes in a microcentrifuge at top speed (>15,000 × *g*) for a maximum of 20 seconds. Do not centrifuge for >20 seconds.

3. Place the tubes in the magnetic stand, then let the tubes stand for 1 minute.

   Note: The Magnetic Particles with the bound DNA/RNA are magnetically captured after approximately 1 minute.

4. Without disturbing the Magnetic Particles, remove the supernatant using a PIPETMAN™ pipette or by aspiration.

5. Remove the tube rack (with tubes) from the magnetic stand, then add 300 µL of Wash Solution to each tube for a second wash. Vortex the tubes for 5 seconds at room temperature at 900 rpm.

6. Centrifuge the tubes in a microcentrifuge at top speed (>15,000 × *g*) for a maximum of 20 seconds. Do not centrifuge for >20 seconds.

7. Place the tubes in the magnetic stand, then let the tubes stand for 1 minute.

   Note: The Magnetic Particles with the bound DNA/RNA are magnetically captured after approximately 1 minute.

8. Open all tubes, then start the 5-minute timer.

9. Without disturbing the Magnetic Particles, remove the supernatant using a PIPETMAN™ pipette or by aspiration.

   Use a P200 to remove the remaining solution from the bottom of the tube.

10. With the tube lid open, air-dry the Magnetic Particles pellet in the magnetic stand for no more than 5 minutes at room temperature.

    IMPORTANT! Air-dry to remove ethanol from the Wash Solution. After dried, the DNA/RNA stays bound to the magnetic beads. Do not over-dry; over-dried beads are not easily resuspended.

Elute the DNA/RNA

1. Add 50 µL of Elution Buffer to each tube.

2. Vortex the tubes for 20 seconds at high speed, then incubate the tubes at 70°C for 7 minutes. Vortex the tubes two to three times during the incubation to help resuspension.

   Note: *(Optional)* If vortexing does not result in full resuspension, then wash the beads off the tube by pipetting.

3. Centrifuge the tubes in a microcentrifuge at top speed (>15,000 × *g*) for a maximum of 20 seconds. Do not centrifuge for >20 seconds.

4. Place the tubes in the magnetic stand, then let the tubes stand for 1 minute.

   Note: The Magnetic Particles with the bound DNA/RNA are magnetically captured after approximately 1 minute.

5. Without disturbing the Magnetic Particles, transfer the liquid phase containing the eluted DNA/RNA to a new nonstick 1.5-mL microcentrifuge tube.

6. Centrifuge the tube at top speed (>15,000 × *g*) for 3 minutes to collect the Magnetic Particles at the bottom, then place the tubes in the magnetic stand for 1 minute.

7. Without disturbing the Magnetic Particles, transfer the liquid phase containing the eluted DNA/RNA to the nonstick 1.5-mL microcentrifuge tube.

   Note: Magnetic Particles can inhibit PCR.

When you finish the sample extraction procedure, see the *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836) or the *ViralSEQ™ Real-Time PCR Kits User Guide* (Pub. No. 4445235) to set up PCR for DNA/RNA quantification.

4 Automated protocol for DNA/RNA extraction

You can use the KingFisher™ Flex or MagMAX™ Express 96-deep well automation platforms to automate the extraction of DNA/RNA. For all chemicals, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Before each use of the kit

Ensure that you have the correct plates

The KingFisher™ Flex or the MagMAX™ Express require 5 plates.

Plate name	Plate type
Lysis	96 deep-well plate
Wash 1	96 deep-well plate
Wash 2	96 deep-well plate
Elution	96 deep-well plate
Comb loading plate	96 deep-well tip comb combined with 96 standard plate

Prepare the plates

Prepare the Wash 1, Wash 2, and Elution plates:

Plate name	Plate type	Volume of buffer to add
Wash 1	96 deep-well plate	300 µL of Wash buffer
Wash 2	96 deep-well plate	300 µL of Wash buffer
Elution	96 deep-well plate	200 µL of Elution buffer

Prepare the lysis plate

In all steps that require pipetting, dispense liquid at bottom center of the wells.

1. Add 100 µL to the appropriate wells of the 96 deep-well Lysis plate:
   - 3 wells for each sample
   - 3 wells for each sample + ERC
   - 3 wells for NEG

   Note: Ensure that **Diluted Yeast tRNA** was added to each plasmid sample. See “(Plasmid samples only) Add Yeast tRNA” on page 13.

2. Add 10 µL of 5 M NaCl to each sample well.
3. Add 70 µL Proteinase K/Proteinase K (PK) Buffer II mix to each sample well.

Process samples on the instrument

1. Select the script or program for the instrument and kit that you are using:

Kit	Instrument	Script
PrepSEQ™ Residual DNA Sample Preparation Kit	KingFisher™ Flex	**PrepSEQ_resDNA_v1**
	MagMAX™ Express-96	**PrepSEQ_resDNA_2011** **PrepSEQ_1hr_resDNA** (if installed)
PrepSEQ™ Nucleic Acid Sample Preparation Kit	KingFisher™ Flex	**PrepSEQ_NucleicAcid_v1**

2. Load the plates into the instrument in the order listed below. After loading each plate, press **START** to move the turntable.
   a. Comb loading plate
   b. Elution plate with 200 µL of Elution Buffer
   c. Wash 2 plate with 300 µL of wash buffer
   d. Wash 1 plate with 300 µL of wash buffer
   e. Lysis plate
3. Press **START** to begin the PK digestion process.

   The instrument mixes the samples for 10 seconds at fast speed, then incubates the samples at 56°C for 30 minutes, mixing at slow speed. When digestion is complete, the instrument pauses and returns the Lysis plate to the loading position.
4. After the digestion step is complete, add additional components to the Lysis plate:
   a. Remove the Lysis plate from the instrument.

b. Add 360 µL of Lysis Solution to each sample well.

c. Add 30 µL of Magnetic Particle suspension to each sample well.

d. Add 400 µL of Binding Solution to each sample well, then immediately pipet up-and-down three times to mix.

e. Place the plate back into the instrument loading position, then press **START** to begin binding.

5. When DNA/RNA extraction is finished, the instrument returns the Elution plate to the loading position.

When you finish the sample extraction procedure, see the *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836) or the *ViralSEQ™ Real-Time PCR Kits User Guide* (Pub. No. 4445235) to set up PCR for DNA/RNA quantitation.

Troubleshooting

Observation	Possible cause	Action
Poor extraction efficiency (low yields)	Overdrying the sample.	Start the 5-minute timer before removing ~300 µL from the first 6–8 samples. Then continue removing wash buffer from the remaining samples.
	Magnetic Particles are difficult to resuspend during the elution.	Incubate the pellets at 70°C for >7 minutes. Vigorously vortex the tubes three times during incubation to help resuspension. Do not overdry. If necessary, repeat the incubation and vortexing steps.
	Formation of precipitate in Magnetic Particles.	Incubate the Magnetic Particle suspension at 37°C with intermittent vortexing at 900 rpm until the particles are completely suspended.
	PK Buffer was used instead of PKII Buffer.	Use PKII Buffer.
Particles no longer produce consistent results (fine brown sandy particles and brown color are observed in the supernatant)	Samples have low pH.	Measure the pH of the sample and adjust the pH to between 6 and 8.
	Magnetic Particles were stored at –20°C.	Order new materials and store them at room temperature.

Good laboratory practices

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Avoiding false positives due to cross-contamination

To avoid false positives due to cross-contamination:

- Prepare and close all negative control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative control, unknown, and positive control samples.

Plate layout suggestions

- For each plate row, dispense in sequence from left to right the: negative controls, unknown samples and ERCs, and positive controls (at the end of the row or column).
- Place positive controls in one of the outer columns (10–12).
- If possible, separate all samples from each other by at least one well; if space is limiting, place at least one well between unknown samples and controls.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! **GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! **HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! **4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication Number
PrepSEQ™ Residual DNA Sample Preparation Kit Quick Reference	4469839
resDNASEQ™ Quantitative DNA Kits User Guide	4469836
resDNASEQ™ Quantitative DNA Kits Quick Reference	4469837
ViralSEQ™ Real-Time PCR Kits User Guide	4445235
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
Applied Biosystems™ MagMAX™ Express 96 User Manual	N07848

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