



# PrepSEQ® Residual DNA Sample Preparation Kit

Catalog Numbers 4413686, 4415414, 4413713, and 4415413

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Revision D



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## **Product Information**

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

### **Product overview**

The PrepSEQ<sup>®</sup> Residual DNA Sample Preparation Kit extracts host-cell DNA from products produced in cell lines such as Chinese hamster ovary (CHO) cells or *E. coli* cells. The kit uses chemical lysis and magnetic beads to efficiently extract genomic DNA from diverse sample types, including samples that contain high protein and low DNA concentration.

To assure accurate quantitative results, Life Technologies protocols call for true triplicate sample preparation and analysis. Extract each test sample in triplicate and perform a single PCR for each extraction. The instrument software calculates a mean quantity and a standard deviation for the triplicate samples, followed by a percent coefficient of variation from this data (SD/Mean Quantity  $\times$  100 = %CV). Based on the method qualification results, you can then assign a %CV to ensure accurate results from each sample tested.

After extraction, you can quantitate the DNA to determine the level of host-cell DNA contamination in the product. For quantitation of CHO host-cell line residual DNA, we recommend use of the resDNASEQ<sup>®</sup> Quantitative CHO DNA Kit as described in the *resDNASEQ*<sup>®</sup> *Quantitative CHO DNA Kit User Guide* (Pub. no. 4415260).

### Kit contents and storage

Components of the PrepSEQ<sup>®</sup> Residual DNA Sample Preparation Kit (Cat. nos. 4413686 and 4415414) include the PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit (Cat. no. 4400799, 3 boxes described in the following table) and the PrepSEQ<sup>®</sup> Residual DNA Sample Preparation Kit (described in the following table).

Reagent	Description	Storage		
PrepSEQ® Nucleic Acid Extraction Kit (Box 1)				
Lysis Buffer	2 bottles, 50 mL/bottle	Store at room temperature.		
Binding Solution (Isopropanol)	1 empty bottle	NA		
Wash Buffer Concentrate	2 bottles, 26 mL/bottle	Store at room temperature.		
Elution Buffer	1 bottle, 25 mL	Store at room temperature.		
Proteinase K (PK) Buffer	1 bottle, 50 mL	Store at room temperature.		
PrepSEQ® Nucleic Acid Ext	raction Kit (Box 2)			
Magnetic Particles	2 tubes, 1.5 mL/tube	Store at 2–8°C.		
PrepSEQ® Nucleic Acid Ext	raction Kit (Box 3)			
Proteinase K (20 mg/mL)	1 tube, 1.25 mL	Store at or below –20°C.		
PrepSEQ® Residual DNA Sa	imple Preparation Kit			
Proteinase K (20 mg/mL)	1 tube, 1.25 mL	Store at or below –20°C.		
Yeast tRNA (10 mg/mL)	1 tube, 0.5 mL	Store at or below –20°C.		
Glycogen (5 mg/mL)	2 tubes, 1.0 mL/tube	Store at or below –20°C.		
Instructions (only included with Cat. no. 4415414)				
User Guide (this document)	Pub. no. 4452598			
Quick Reference Card	Pub. no. 4415258			

The PrepSEQ $^{\circledR}$  Residual DNA Sample Preparation Kit can also be purchased along with the resDNASEQ $^{\circledR}$  Quantitative CHO DNA Kit (Cat. nos. 4413713 [kits] and 4415413 [kits with protocols and quick reference cards]).

## Automation instrument, plastics, and accessories

 $MagMAX^{\text{\tiny{TM}}}$  Express-96 Deep Well Magnetic Particle Processor (Cat. no. 4400079) accessories include:

Item	Cat. no.
MagMAX <sup>™</sup> Express-96 Deep Well (DW) plate	4388476
MagMAX <sup>™</sup> Express-96 Deep Well (DW) well tip combs	4388487
MagMAX <sup>™</sup> Express-96 Deep Well (DW) magnetic head	4388435
MagMAX <sup>™</sup> Express-96 Deep Well (DW) standard plates	4388475
Magnetic Stand-96	AM10027
Vortex Adapter-60, for use with the Vortex-Genie® mixer	AM10014

## Materials required but not included

Item	Source or cat. no.
Equipment	
Two block heaters, for use with 2-mL tubes, 56°C and 70°C	Major laboratory supplier (MLS)
<b>Note:</b> If only 1 block heater is available, set the temperature to 56°C, then reset to 70°C after the 56°C incubation. If 2 block heaters are available, set one to 56°C and the other to 70°C.	
Magnetic stand, 16-position	4457858
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® Mixer	AM10014

Item	Source or cat. no.
Consumables	
Disposable gloves	MLS
Aerosol-resistant micropipette tips	MLS
Pipetman <sup>®</sup> Pipettors, P1000, P200, P20 and P10: • Positive-displacement	MLS
Air-displacement	
Multichannel	
Pipettes	MLS
Microcentrifuge tubes, nonstick, RNase-free, 1.5-mL, 1 box (250 tubes/box)	AM12450
Safe-Lock PCR clean microcentrifuge tubes, round-bottom, 2-mL	VWR 62111-754
Reagents	
Ethanol, 95%	MLS
<b>IMPORTANT!</b> Do not use denatured ethanol. It contains components that are not compatible with the protocol.	
Isopropanol, 100%	MLS
5M NaCl and 1N NaOH solutions	MLS
Hydrochloric acid (HCI)	MLS



## PrepSEQ® Residual DNA Sample Preparation Kit

### Reagent preparation

Before you use the PrepSEQ<sup>®</sup> Residual DNA Sample Preparation Kit, prepare the following solutions:

- PrepSEQ® Binding Solution:
  - a. Add 30 mL of 100% isopropanol to the Binding Solution bottle.
  - b. Label the bottle to indicate that it contains isopropanol, then store the bottle at ambient temperature.
- PrepSEQ<sup>®</sup> Wash Buffer Concentrate:
  - a. Add 74 mL of 95% ethanol to the bottle that is labeled PrepSEQ<sup>®</sup> Wash Solution Concentrate, then mix completely.
  - b. Label the bottle to indicate that it contains ethanol, then store the bottle at ambient temperature.
- Lysis Solution Mix of Lysis Buffer, tRNA, and glycogen, prepared immediately prior to starting sample preparation:
  - Prepare a fresh mixture according to the following table.

Reagent	Volume (µL)
Glycogen (5 mg/mL)	180
tRNA (10 mg/mL)	4
Lysis buffer	7600
Total	7784

- Use 360 μL of the mix for sample preparation per 100 μL of sample.
- Proteinase K/Proteinase K Buffer mix:
  - Prepare a mix that contains Proteinase K and Proteinase K buffer for the total number of samples to be processed.
  - Include a 10% overage to account for pipetting losses. For example, if you have 9 samples, create a mix for 10 samples as shown in the following table. Then add 70  $\mu$ L of the mix per 100  $\mu$ L of sample.

1 reaction (per 100 µL of sample		10 reactions (per 100 µL of sample)	
Proteinase K	10 μL	100 μL	
Proteinase K buffer	60 μL	600 μL	
Total	70 μL	700 μL	

### • Magnetic particles

- a. Immediately before using, incubate the tube containing the magnetic particles at  $37^{\circ}$ C for 10 minutes.
- b. If necessary, use a P1000 Pipetman<sup>®</sup> pipette to agitate the particles at the bottom of the tube before vortexing. Small aggregations of particles will reduce performance.
- c. Vortex the tube at setting #7 to completely resuspend the particles.

### Extract host cell DNA

To extract host-cell DNA from products that are produced in cell lines such as CHO or *E. coli*, use the following workflow.

## Manual residual DNA extraction workflow

The manual extraction workflow is shown below. For details, go to page 12.

### Prepare digestion reaction tubes and Proteinase K reaction

Step 1: Label 2-mL, safe-lock tubes as appropriate, then add 100–200  $\mu L$  of sample to each tube.

 $\blacksquare$ 

**Step 2**: Adjust pH level to between 6 and 8, first using 10N NaOH or 10N HCl (if necessary), then measure and confirm the pH level. The required volume depends on the sample pH. Adjust NaCl concentration to approximately 0.5M (if necessary).

Step 3: Make a master mix of Proteinase K Buffer and
Proteinase K, then add 70 μL of Proteinase K Buffer/Proteinase K to the sample per 100 μL of sample. Briefly vortex and spin. Incubate at 56 °C for 30 min.

Step 4: Add 360  $\mu$ L of lysis solution mix per 100  $\mu$ L of starting

### **Bind DNA**

**Step 1**: Incubate the Magnetic Particles at 37°C for 10 min, then vortex the Magnetic Particles at #7 to completely resuspend particles.

 $\blacksquare$ 

Step 2: Add 30  $\mu$ L of Magnetic Particles using a wide-bore pipette tip.

Step 3: Add 300 µL of Binding Solution per 100 µL of starting sample, invert twice, then vortex for 5 min at setting #7.

**Step 4**: Spin for 15 sec, place the tubes into a magnetic stand for 5 min or until the solution is clear, then remove and discard the supernatant.

### Wash DNA

Step 1: Remove tubes from the magnetic stand, then add 300  $\mu$ L of Wash Solution. Invert the tubes twice. Vortex for 5 sec at setting #7 (see the corresponding IMPORTANT on page 13).

 $\blacksquare$ 

**Step 2**: Spin for 15 sec, then place the tubes into the magnetic stand for 1 min (see the corresponding Note on page 13).

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**Step 3**: Without disturbing the magnetic beads, remove and discard the supernatant using a Pipetman<sup>®</sup> pipette or by aspiration.

 $\blacksquare$ 

**Step 4**: Remove tubes from the magnetic stand, then add 300 µL of Wash Solution. Invert the tubes twice. Vortex for 5 sec at setting #7 (see the corresponding IMPORTANT on page 13).

**Step 5**: Spin for 15 sec, then place the tubes into the magnetic stand for 1 min (see the corresponding Note on page 13).

**Step 6**: Without disturbing the magnetic beads, remove and discard the supernatant using a Pipetman<sup>®</sup> pipette or by aspiration.

Step 7: Use a P200 pipettor to remove residual solution.

**Step 8**: Leave the tube lids open for 5 min to air dry (see the corresponding IMPORTANT on page 13).

### **Elute DNA**

Step 1: Add 50  $\mu L$  of Elution Buffer to each tube (see the corresponding IMPORTANT on page 13).

 $\blacksquare$ 

**Step 2**: Vortex for 10 sec at high speed, then incubate the tubes at 70°C for 7 min. Vortex 2–3 times to resuspend particles.

 $\blacksquare$ 

**Step 3**: Spin for 15 sec and place the tubes into the magnetic stand for 2 min. Then transfer the eluate to a nonstick 1.5-mL tube.

**Step 4**: Spin for 3 min at top speed. Place the tubes into a magnetic stand.

**Step 5**: Transfer the eluate to a nonstick 1.5-mL tube. Avoid the magnetic beads (see the corresponding Note on page 14).

 $\blacksquare$ 

When done, set up PCR using 10  $\mu$ L of eluate (see the resDNASEQ® Quantitative CHO DNA Kit User Guide, Pub. no. 4415260).

## Manually extract residual DNA

- 1. Prepare the digestion reaction tubes and Proteinase K reaction.
  - a. Label 2-mL Safe-Lock tubes as appropriate, then add 100–200  $\mu L$  of sample into each tube.

**Note:** Test samples from the early purification process often contain levels of DNA that are above the CHO residual DNA assay standard curve's highest point. Refer to "Dilute residual DNA samples (optional)" on page 14. Alternatively, make dilutions of the extracted DNA before running the PCR.

- **b.** Add an Extraction Negative Control (use 100  $\mu$ L or 200  $\mu$ L DNA Dilution Buffer as sample).
- **c.** If necessary, adjust the pH with 10 N NaOH or 10 N HCl to achieve the optimal pH value of between 6 and 8 for the sample.
  - The optimal pH value of the sample for DNA extraction is between 6 and 8. When necessary, adjust the pH with 10 N NaOH or 10 N HCl. The amount of NaOH or HCl to adjust pH can be determined first with a larger volume of sample. The pH can be measured with pH paper. For example, if 20  $\mu L$  of 10 N NaOH is required to adjust a pH of 1 mL of sample to higher than 6, then 2  $\mu L$  can be added to 100  $\mu L$  of sample before sample preparation. Or 100  $\mu L$  of the already adjusted sample can be used for sample preparation.
- d. Adjust salt concentration in the samples with 5 M NaCl to approximately 0.5 M, if the salt concentration is lower than 0.5 M. For example, add 10  $\mu L$  of 5 M NaCl per 100  $\mu L$  of sample.
- e. Make a master mix of Proteinase K buffer and Proteinase K, then add 70  $\mu$ L of Proteinase K buffer/Proteinase K per 100  $\mu$ L of sample. Briefly vortex and spin. Incubate at 56°C for 30 minutes.
- f. Add 360  $\mu$ L of freshly made Lysis Solution Mix per 100  $\mu$ L of starting sample (see "Reagent preparation" on page 9).

### 2. Bind the DNA.

- **a.** Incubate the Magnetic Particles at 37°C for 10 minutes, then vortex the Magnetic Particles at setting #7 until resuspension is complete.
- **b.** Add 30  $\mu$ L of the Magnetic Particles to 100–200  $\mu$ L of sample using a widebore pipette tip.
- c. Add 300  $\mu$ L of the Binding Solution per 100  $\mu$ L of starting sample, close the caps and invert the tubes twice to mix, then vortex the tubes for 5 minutes at setting #7.
- d. Quick-spin the tubes in a microcentrifuge for 15 seconds to pellet the Magnetic Particles, then place the tubes in the magnetic stand and let the tubes stand for 5 minutes or until the solution is clear. Without disturbing the magnetic beads, remove and discard the supernatant using a Pipetman<sup>®</sup> pipette or by aspiration.

### 3. Wash the DNA.

a. Remove the tubes from the magnetic stand, then add 300  $\mu$ L of Wash Solution to the tubes. Invert the tubes back and forth twice. Vortex the tubes for 5 seconds at room temperature, at setting #7.

**IMPORTANT!** If the Magnetic Particles stick to the tube wall, flush gently with the Wash Solution to detach the particles.

**b.** Quick-spin the tubes in a microcentrifuge for 15 seconds, then place the tubes in the magnetic stand and let the tubes stand for 1 minute.

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

- **c.** Without disturbing the magnetic beads, remove and discard the supernatant using a Pipetman<sup>®</sup> pipette or by aspiration.
- d. Remove tubes from the magnetic stand, then add 300  $\mu$ L of Wash Solution to the tube. Invert the tubes back and forth twice. Vortex the tubes for 5 seconds at room temperature, at setting #7.

**IMPORTANT!** If the Magnetic Particles stick to the tube wall, flush gently with the Wash Solution to detach the particles.

**e.** Quick-spin the tube in a microcentrifuge for 15 seconds, then place the tubes in the magnetic stand and let the tubes stand for 1 minute.

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

- f. Without disturbing the magnetic beads, remove and discard the supernatant using a Pipetman<sup>®</sup> pipette or by aspiration.
- **g.** Use a P200 to remove the residual solution from the bottom of the tube.
- h. With the tube lid open, air-dry the Magnetic Particle pellet in the magnetic stand for 5 minutes at room temperature.

**IMPORTANT!** Air-drying removes ethanol from the Wash Solution. Ethanol decreases recovery and inhibits PCR.

### 4. Elute the DNA.

a. Add 50  $\mu L$  of Elution Buffer to each tube containing Magnetic Particles that have bound DNA.

**IMPORTANT!** The Magnetic Particles may be difficult to detach from the tube wall. Place the tube in the microcentrifuge with the Magnetic Particle pellet oriented toward the center, then spin the tube for 30 seconds to detach the Magnetic Particles from the tube wall into the Elution Buffer. If the Magnetic Particles are difficult to resuspend, use a P200 to gently pipet up and down several times. Be careful not to let the Magnetic Particles stick inside the pipette tip.

- **b.** Vortex the tubes for 10 seconds at high speed, then incubate the tubes at 70°C for 7 minutes. Vortex the tubes 2–3 times during the incubation to help resuspension.
- c. Centrifuge the tubes in a microcentrifuge for 15 seconds and place the tubes in the magnetic stand and let the tubes stand for 2 minutes. Then transfer the liquid phase containing the eluted DNA to a new nonstick 1.5-mL microcentrifuge tube.

**Note:** The Magnetic Particles are magnetically captured in approximately 1 minute. DNA is in the eluate.

- **d.** Centrifuge the tube at top speed (>15,000  $\times$  g) for 3 minutes to pellet the residual Magnetic Particles, then place the tube in the magnetic stand and let the tube stand for 1 minute.
- e. Transfer the liquid phase containing the eluted DNA to the nonstick 1.5-mL microcentrifuge tube without disturbing the Magnetic Particles. Use 10 µL of the eluate in the real-time PCR.

**Note:** Magnetic particles can inhibit PCR.

When you finish the sample extraction procedure, refer to the *resDNASEQ*® Quantitative CHO DNA Kit User Guide (Pub. no. 4415260) to set up PCR for DNA quantitation.

### Dilute residual DNA samples (optional)

Test samples from the early purification process often contain levels of DNA that are above the CHO residual DNA assay standard curve's highest point. You must dilute these samples (from 1/100 up to 1/10,000) prior to PrepSEQ® Residual DNA sample preparation.

Diluting samples in water is often not efficient because the PrepSEQ® Residual DNA Sample Preparation Kit protocol is optimized for highly efficient recovery of DNA from complex mixtures of proteins, buffer, and salts.

To rectify this situation, dilute test samples prior to DNA extraction and purification by using the following solution:

Sample dilution buffer 1X PBS (pH 7.4; free of Mg and Ca) plus 0.5 M NaCl (1X PBS can be made from 10X PBS, Cat. no. AM9624).

If the sample is being diluted, use the sample dilution buffer as the negative process control instead of water.

Alternatively, dilute extracted DNA before running the PCR.

### Automated protocol for residual DNA extraction

You can use the MagMAX<sup>™</sup> Express automation platform to automate the extraction of host cell line residual DNA.

### **Automated CHO** DNA extraction workflow

The automated extraction workflow is shown below. For details, go to page 16.

### Prepare the plates

Prepare the lysis, Wash 1, Wash 2, Elution and Comb loading plates according to the table on page 16.



### Select the instrument protocol

Select the program labeled **PrepSEQ\_ResDNAv1** from the MagMax<sup>™</sup> Express.



### Load the plates

Step 1: Press START, then position the plates according to the Display window instructions.

- a. Comb loading plate
- b. Elution plate with 100-200 µL elution buffer
- c. Wash 2 plate with 300 µL wash buffer
- d. Wash 1 plate with 300 µL wash buffer
- e. Lysis plate



### Prepare samples for digestions

**Step 1**: Add 100  $\mu$ L of sample to a well of the 96 deepwell plate. Adjust pH level to between 6 and 8, first using 10 N NaOH or 10 N HCl (if necessary), then measure and confirm the pH level. Adjust NaCl concentration to approximately 0.5 M (if necessary).



**Step 3**: Place the plate in the processor, then press **START** to begin the lysis process.



**Step 2**: Make a master mix of Proteinase K buffer and Proteinase K, then add 70 uL to each sample. Vortex and spin briefly. Incubate the mix at 56°C for 30 min.

The instrument mixes the samples for 10 sec at fast speed, then incubates the samples at 57°C for 30 min, mixing at slow speed.



### Prepare the lysis and bind the DNA

**Step 1**: Remove the plate from the instrument, then add 360  $\mu$ L of Lysis Solution using a multi-channel pipette. Pipet up and down 2 times to mix.



**Step 4**: Add 300 µL of Binding Solution using an 8-channel pipette, then pipet up and down 2 times.



**Step 2**: Incubate the Magnetic Particle suspension at 37°C for 10 min, then vortex for 2 min or until completely suspended.



**Step 5**: Place the plate back into the instrument loading position, then press **START** to begin binding..



The instrument mixes the beads for 15 min (superfast speed), collects beads (45 counts), then washes and elutes the DNA.

**Step 3**: Add 30 µL of Magnetic Particle suspension to the sample, then shake the plate gently to mix.

 $\blacksquare$ 

### Measure the eluate volume

Step 1: Place the Elution plate on a Magnetic Stand-96 to attract residual particles to the bottom of the wells.



**Step 2**: Use a pipette to measure the eluate volume from several wells (eluate volumes can vary). The average eluate volume is used to calculate recovery efficiency.



Step 3: Use a multi-channel pipette to carefully transfer 10 µL of eluate to the PCR plate. Do not touch particles.

### Prepare for automated sample preparation of CHO DNA

1. Prepare the plates.

Plate name	Plate type	Sample or buffer added	
Lysis	96 deep-well plate	100 μL sample, 60 μL PK buffer, 10 μL PK	
Wash 1	96 deep-well plate	300 μL Wash buffer	
Wash 2	96 deep-well plate	300 μL Wash buffer	
Elution	96 deep-well plate	100 or 200 μL Elution buffer	
Comb loading plate	96 deep-well tip comb combined with 96 standard plate	NA	

- 2. Select the instrument program labeled **PrepSEQ\_ResDNAv1** from the MagMax<sup>™</sup> Express.
- **3.** Load plates into the instrument, in the order listed here. After loading each plate, press **START** to move the turntable.
  - a. Comb loading plate
  - **b.** Elution plate with 100–200 μL buffer
  - c. Wash 2 plate with 300 µL wash buffer
  - **d.** Wash 1 plate with 300 μL wash buffer
  - e. Lysis plate
- 4. Prepare samples for digestion.
  - a. Add 100  $\mu$ L of sample to a well of the 96 deep-well plate. If necessary, adjust pH level using 10 N NaOH or 10 N HCl (optimum range is 6 to 8), then measure to confirm the pH level.

Optional: Add 10  $\mu L$  of 5 M NaCl if the salt concentration of the sample is lower than 0.5 M.

**Note:** If necessary, centrifuge the plate at 1000 rpm for 1 minute, in a regular bench-top microcentrifuge, to spin down the solution on the wall.

- b. Make a master mix of Proteinase K buffer and Proteinase K, then add 70  $\mu$ L to each sample. Briefly vortex, then briefly spin the master mix. Incubate the mix at 56°C for 30 minutes.
- c. Place the plate in the processor, then press START to begin the lysis process. The instrument mixes the samples for 10 seconds at fast speed, then incubates the samples at 57°C for 30 minutes, mixing at slow speed.
- **5.** Prepare the lysis and bind the DNA.
  - a. Remove the plate from the instrument, then add 360  $\mu$ L of Lysis Solution using an 8-channel pipette. (The Lysis Solution will be freshly supplemented with Glycogen and Yeast tRNA at final concentrations of 8  $\mu$ g/ $\mu$ L and 10  $\mu$ g/ $\mu$ L, respectively, as described on page 9). Pipet up and down 2 times to mix.
  - **b.** Incubate the Magnetic Particle suspension at 37°C for 10 minutes, then vortex for 2 minutes or until completely suspended.
  - c. Add 30  $\mu$ l of Magnetic Particle suspension to the sample, then shake the plate gently to mix.

- **d.** Add 300 μl of Binding Solution using an 8-channel pipette, then pipet up and down 2 times to mix.
- **e.** Place the plate back into the instrument loading position, then press **START** to begin binding.

The instrument mixes the beads for 15 minutes (superfast speed), collects beads (45 counts), then washes and elutes the DNA. The comb with beads will automatically be washed in the Wash 2 plate and placed on the comb loading plate. Then the Elution plate will be automatically placed into its loading position and the eluates will be ready for analysis.

### **6.** Measure the eluate volume.

- **a.** Place the Elution plate on a Magnetic Stand-96 to attract residual particles to the bottom of the wells.
- **b.** Use a pipette to measure the eluate volume from several wells (eluate volumes can vary). The average eluate volume is used to calculate recovery efficiency.
- c. Use a multi-channel pipette to carefully transfer 10  $\mu$ L of eluate into the PCR plate for the real-time PCR assay. Do not touch the particles.

## **Troubleshooting**

Observation	Possible cause	Action
Poor extraction efficiency (low yields)	Ethanol is in the Wash Solution (step h on page 13).	Thoroughly air-dry the Magnetic Particles pellet in the magnetic stand for 5 minutes at room temperature.
	Magnetic Particles are attached too tightly to the tube wall during the elution (step a on page 13).	Place the tube in the microcentrifuge with the Magnetic Particles pellet oriented toward the center. Spin the tube for 30 seconds to detach the Magnetic Particles from the tube wall into the Elution Buffer.
	Magnetic Particles are difficult to resuspend during the elution (step b on page 14).	Incubate the pellets at 70°C for 7 minutes. Vortex the tubes 3 times during incubation to help resuspension.
	Formation of precipitate in Magnetic Particles (page 14).	Incubate Magnetic Particles at 37°C for 10 minutes, then vortex the Magnetic Particles at setting #7 for 30 seconds to completely resuspend the particles.
Particles no longer produce consistent	Samples have low pH (step c on page 12).	Measure pH of the sample and adjust pH to between 6 and 8.
results (fine brown sandy particles and brown color in the supernatant)	Magnetic Particles were stored at -20°C ("Kit contents and storage" on page 6, "Magnetic Particles" on page 6).	Order new materials and store at 4°C.

## 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, etc). 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, and 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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- 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.

## Appendix A Safety Biological hazard safety

## Specific chemical handling

CAS	Chemical	Phrase
593-84-0	Guanidine Isothiocyanate	Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

### **Biological hazard safety**



**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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may 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), 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf.
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: www.who.int/csr/resources/ publications/biosafety/Biosafety7.pdf.

## **Documentation and Support**

### Related documents

For additional documentation see "Obtaining support" on page 22.

- For information on performing PCR after sample extraction, refer to the resDNASEQ® Quantitative CHO DNA Kit v3.0 User Guide (Pub. no. 4415260).
- For information on MagMAX<sup>TM</sup> Express-96 DW instrument, see the user guide available at the web catalog page for the instrument at www.lifetechnologies.com.

Portable document format (PDF) versions of this guide and the documents shown above are available at **www.lifetechnologies.com**.

### **Obtaining SDSs**

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

**Note:** For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

### **Obtaining Certificates of Analysis**

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

### **Obtaining support**

For the latest services and support information for all locations, go to:

### www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

### Limited product warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.







