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# PrepSEQ® Residual DNA Sample Preparation Kit

**Catalog Numbers** 4413686 (kit), 4415414 (kit with protocol and quick reference card), 4413713 (combo kit†), and 4415413 (combo kit with protocol and quick reference card)

**Publication Number** 4415258 **Revision** C

**Note:** For safety and biohazard guidelines, refer to the "Safety" section in the *PrepSEQ® Residual DNA Sample Preparation Kit User Guide* (Pub. no. 4415259). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Product overview

The PrepSEQ® 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.

## Kit contents and storage

| Reagent | Quantity | 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 Extraction Kit (Box 2)** | | |
| Magnetic Particles | 2 tubes, 1.5 mL/tube | Store at 2–8 °C. |
| **PrepSEQ® Nucleic Acid Extraction Kit (Box 3)** | | |
| Proteinase K (20 mg/mL) | 1 tube, 1.25 mL | Store at or below –20°C. |
| **PrepSEQ® Residual DNA Sample 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. |

† combo = PrepSEQ® Residual DNA Sample Preparation Kit + resDNASEQ® Quantitative CHO DNA Kit

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## Automation instrument, plastics, and accessories

MagMAX™ Express-96 Deep Well Magnetic Particle Processor (Cat. no. 4400079) accessories include:

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

## Manual sample preparation of CHO DNA

### Overview

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 then 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 × 100 = %CV). Based on the method qualification results, you can then assign a %CV to ensure accurate results from each sample tested.

### Reagent preparation

Before you use the PrepSEQ® 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® Wash Buffer Concentrate:
  a. Add 74 mL of 95% ethanol to the bottle that is labeled PrepSEQ® 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 µL of the mix per 100 µL 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°C for 10 minutes.
  b. If necessary, use a P1000 Pipetman® 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.

## Manual residual DNA extraction workflow

**Prepare digestion reaction tubes and Proteinase K reaction**

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

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**Step 2**: 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. The required volume depends on the sample pH. Adjust NaCl concentration to approximately 0.5 M (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.

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**Step 4**: Add 360 µL of lysis solution mix per 100 µL of starting sample.

▼

**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.

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**Step 2**: Add 30 µL 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 µL of Wash Solution. Invert the tubes twice. Vortex for 5 sec at setting #7.

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**Step 2**: Spin for 15 sec, then place the tubes into the magnetic stand for 1 min.

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

▼

**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.

**Step 5**: Spin for 15 sec, then place the tubes into the magnetic stand for 1 min.

▼

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

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

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**Step 8**: Leave the tube lids open for 5 min to air dry.

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**Elute DNA**

**Step 1**: Add 50 µL of Elution Buffer to each tube.

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**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.

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**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.

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**Step 5**: Transfer the eluate to a nonstick 1.5-mL tube. Avoid the magnetic beads.

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

## Automated sample preparation of CHO DNA

Before you begin the automated sample preparation process, use the table below to prepare the plate. Then refer to the automated CHO DNA detection workflow that follows.

### Prepare the plate

| 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 |

### Select the instrument protocol

Select the program labeled **PrepSEQ_ResDNAv1** from the MagMAX™ Express.

## Automated CHO DNA extraction workflow

**Prepare the plates**

Prepare the lysis, Wash 1, Wash 2, Elution and Comb loading plates.

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**Select the instrument protocol**

Select the program labeled **PrepSEQ_ResDNAv1** from the MagMax™ Express.

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**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 µL of sample to a well of the 96 deep-well 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 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.

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

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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.

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**Prepare the lysis and bind the DNA**

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

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

▼

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

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

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

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

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**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.

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**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.

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

## Limited product warranty

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## Notes



**Headquarters**

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

**For support visit** lifetechnologies.com/support

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