

PrepSEQ™ Residual DNA Sample Preparation Kit

Note: For safety and biohazard guidelines, refer to the “Safety” section in the PrepSEQ™ Residual DNA Sample Preparation Kit Protocol (PN 4415259). For every chemical, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product overview

The PrepSEQ™ Residual DNA Sample Preparation Kit Protocol 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

The PrepSEQ™ Residual DNA Sample Preparation Kit contains the PrepSEQ™ Nucleic Acid Extraction Kit (PN 4400799) and the PrepSEQ™ Residual DNA Sample Preparation Kit (PN 4399042). Kit components include:

Reagent	Description	Storage	Part number
PrepSEQ Nucleic Acid Extraction Kit (Box 1)			4400793
Lysis Buffer	2 bottles, 50 mL/bottle	Store at room temperature.	4400659
Binding Solution (Isopropanol)	1 empty bottle	NA	4400789
Wash Buffer Concentrate	2 bottles, 26 mL/bottle	Store at room temperature.	4400783
Elution Buffer	1 bottle, 25 mL	Store at room temperature.	4400784
Proteinase K (PK) Buffer	1 bottle, 50 mL	Store at room temperature.	4400787
PrepSEQ Nucleic Acid Extraction Kit (Box 2)			4400795
Magnetic Particles	2 tubes, 1.5 mL/tube	Store at 2 to 8 °C.	4401405
PrepSEQ Nucleic Acid Extraction Kit (Box 3)			4400675
Proteinase K	1 tube, 20 mg/mL, 1.25 mL	Store at or below –20 °C.	4403958
PrepSEQ™ Residual DNA Sample Preparation Kit			4399042
Proteinase K	1 tube, 20 mg/mL, 1.25 mL	Store at or below –20 °C.	4403958
Yeast tRNA	1 tube, 10 mg/mL, 0.5 mL	Store at or below –20 °C.	4404626
Glycogen	2 tubes, 5 mg/mL, 1.0 mL/tube	Store at or below –20 °C.	4404627

Automation instrument, plastics, and accessories

MagMAX™ Express-96 DW instrument (PN 4400079) accessories include:

Item	Source or part number
MagMAX™ Express-96 DW plate	4388476
MagMAX™ Express-96 DW well tip combs	4388487
MagMAX™ Express-96 DW magnetic head	4388435
MagMAX™ Express-96 DW standard plates	4388475
Magnetic Stand-96	AM10027
Vortex Adapter-60, for use with the Vortex-Genie	AM10014

Manual sample preparation of CHO DNA

Overview

To assure accurate quantitative results, Applied Biosystems protocols call for true triplicate sample preparation and analysis. Extract each test sample in triplicate and perform a single PCR reaction 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

(SD/Mean Quantity × 100 = % CV)

from this data. 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

- 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® 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 or 200 μ L of sample to each tube.

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 0.5 M (if necessary).

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

Step 4: Add 360 μ L of lysis solution mix per 100 μ L 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.

Step 2: Add 30 μ L of Magnetic Particles using a wide bore pipette tip.

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

Step 4: Spin for 15 seconds, 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 μ L of Wash Solution. Invert the tubes twice. Vortex for 5 sec at setting #7.

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

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

Step 4: Remove tubes from the magnetic stand, then add 300 μ L 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 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.

Elute DNA

Step 1: Add 50 μ L of Elution Buffer to each tube.

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

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.

When done, set up PCR using 10 μ L of eluate (see the *resDNASEQ™ Quantitative CHO DNA Kit Protocol*).

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.

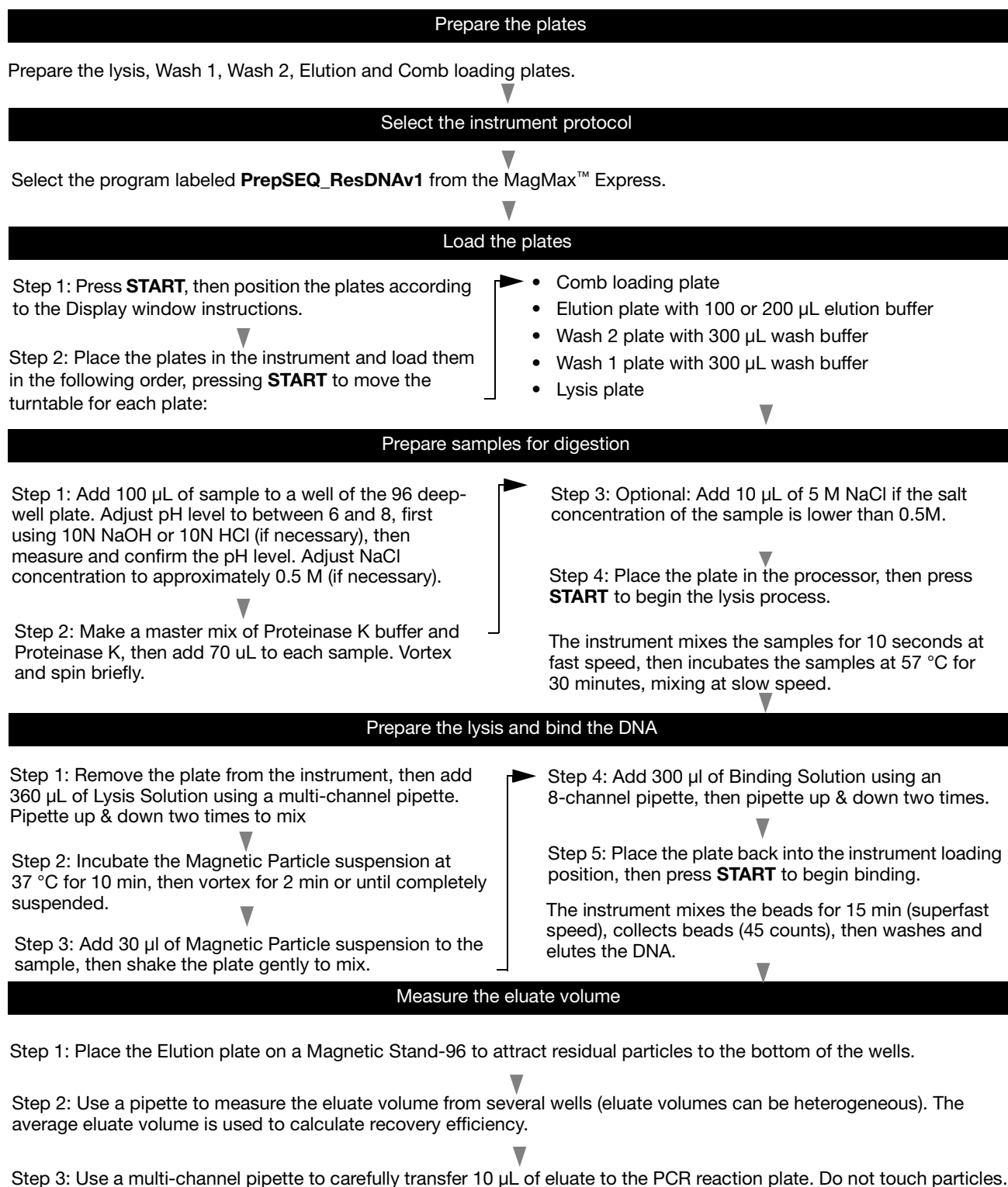
Prepare the plate

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



Notes

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10/2009

