Automated Purification of Residual DNA Using the PrepSEQ™ Residual DNA Sample Preparation Kit and the MagMAX™ Express 96 Instrument

Introduction

Biopharmaceuticals produced in live cell systems such as bacteria and eukaryotic cell lines contain some level of host cell DNA at the time the product is harvested from bioreactors. The host DNA constitutes a potential threat to human health, and manufacturing regulations stipulate that host DNA must be below a certain level in each dose. DNA removal is achieved by sequential purification steps, and accurate quantification of residual DNA at each step is essential in evaluating how efficiently the DNA is being removed and in characterizing the final bulk drug product. Quantitative and consistent recovery of DNA from challenging matrices, including samples that have high protein concentration, low pH, or high salt, presents a technical challenge.

The PrepSEQ™ Residual DNA Sample Preparation Kit can be used in manual DNA purification workflows and now is adapted for higher-throughput workflows (up to 96 sample extractions in two hours) when used in combination with the MagMAX™ Express 96 Magnetic Particle Processor. The special formulation of magnetic particles provided in the PrepSEQ™ Kit ensures highly efficient, specific recovery of DNA from challenging samples.

The MagMAX™ Express 96 Magnetic Particle Processor is designed for automated transfer and processing of magnetic particles in a microplate format. The patented technology of the MagMAX™ Express 96 processor employs 96-well plates and magnetic rods covered with a specially designed disposable tip comb. Before starting the magnetic particle processing, the samples and reagents are dispensed into the plates, and the tip comb is placed onto the MagMAX™ Express 96 Standard Plate (the tip comb is automatically loaded from the standard plate when the run begins). The instrument utilizes rods to collect magnetic beads from solution, then releases the beads into the well containing reagents for the next step of isolation (Figure 1). The effectiveness of bead collection and transfer leads to superior washing, efficient elution, and rapid processing.

This Application Note describes DNA purification using the MagMAX™ Express 96 Magnetic Particle Processor. To create test samples, quadruplicate aliquots of a solution containing 100 mg/mL of IgG were spiked with different amounts of CHO DNA. DNA was then purified from the samples using the automated sample preparation procedure and quantified using real-time PCR and the components of the resDNASEQ™ Quantitative CHO DNA Kit.

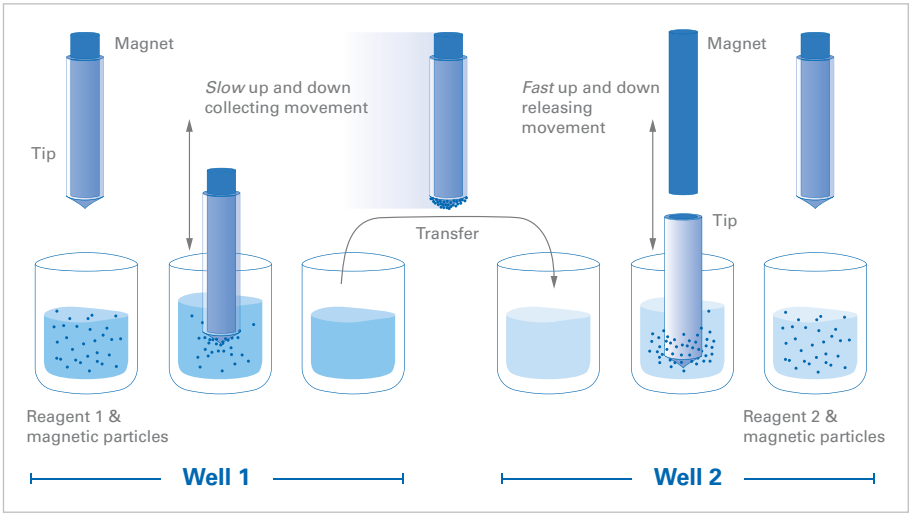

Figure 1. Magnetic Particle Processing on the MagMAX™ Express 96 Processor.

Procedure

Creating CHO DNA Dilutions for Standard Curve and Spike

CHO DNA (3 ng/µL) was 10-fold serially diluted in DNA Dilution Solution (using Ambion's non-stick tubes) to obtain the following concentrations: 0.3 ng/µL, 30.0 pg/µL, 3.0 pg/µL, 0.3 pg/µL, 30.0 fg/µL, 3.0 fg/µL, and 0.3 fg/µL (Figure 2). To generate a standard curve for quantification, a 10 µL aliquot of each of the DNA dilutions was amplified in parallel with the test samples in a real-time PCR assay.

To generate a series of DNA test samples, 3-fold dilutions were made to the 30.0 pg/µL, 3.0 pg/µL, 0.3 pg/µL, and 30.0 fg/µL CHO DNA standard curve solutions to obtain 10 pg/µL, 1 pg/µL, 0.1 pg/µL, and 10 fg/µL solutions, respectively. A 10 µL aliquot of each of these test samples was added to 90 µL of a solution containing 3% mannitol, 2% sucrose, 10 mM L-arginine, 0.01% Tween 20, and 100 mg/mL IgG, so that the final amounts of CHO DNA in each of the test samples were 100 pg, 10 pg, 1 pg, and 0.1 pg, respectively.

Quadruplicates of each test sample were created, extracted, and quantified in parallel.

Overview of the Automated DNA Purification Procedure

100 µL of test sample solution was added to the wells of a 96 deep well plate. 10 µL of diluted CHO DNA was added to get final DNA amounts of 100 pg, 10 pg, 1 pg, and 0.1 pg. 60 µL of proteinase K buffer and 10 µL of proteinase K were then added to the wells. To adjust the salt concentration of the samples to ~0.5 M NaCl, 10 µL of 5 M NaCl was added to each sample. The plate was then placed into the instrument for 30 minutes at 56˚C to allow the proteinase K reaction to proceed.

After proteinase K treatment, the plate was removed from the instrument. Lysis Solution (containing yeast tRNA and glycogen), magnetic particles, and binding solution were added to the wells. The plate was placed back into the instrument where DNA capture, wash, and elution were performed automatically. DNA was eluted with 200 µL of Elution Buffer.

The detailed procedure for automated sample preparation is provided below.

Before starting the DNA purification procedure, prepare the solutions listed below.

Binding Solution: Add 30 mL of 100% isopropanol to the Binding Solution Bottle of PrepSEQ™ kit. Mark the label to indicate that isopropanol was added, then store at ambient temperature.

Wash Solution: Add 74 mL of 95% ethanol to the bottle labeled PrepSEQ™ Wash Solution Concentrate, and mix well. Mark the label to indicate that ethanol was added, then store at ambient temperature.

Lysis Solution: Prepare this solution in a 50 mL Falcon tube by adding 180 µL of PrepSEQ™ Glycogen (15 mg/mL), 4 µL of PrepSEQ™ Yeast tRNA (10 mg/mL), and 7,600 µL of PrepSEQ™ Lysis Buffer. Note: the Lysis Solution needs to be made fresh each day.

Magnetic Particles: To maximize the performance of the magnetic particles, incubate the bottle containing the magnetic particles at 37˚C for 5 to 10 minutes. Just before use, vortex the bottle to completely resuspend the particles.

DNA Purification Protocol

Setting Up the Instrument and Sample Plates

1. Create or select the MagMAX™ Express program for sample preparation (refer to the instrument user manual for help with this step if necessary).
2. Take four 96 Deep Well Plates and label them as follows: Lysis, Wash 1, Wash 2, and Elution. One 96 Deep Well Tip Comb combined with a 96 Standard Plate will also be used in this procedure.

3. Plan the layout of the Lysis plate. Elution and Wash solutions need to be pipetted into the corresponding wells in the Elution and Wash plates.
4. Add 300 µL of Wash Solution to the wells in the Wash 1 and Wash 2 plates, in wells that correspond to the positions of the test samples.
5. Add 200 µL (or 100 µL) of Elution Buffer to wells that will be used for sample preparation in the Elution plate. **Note:** The volume you choose depends on whether you want to elute in 100 µL or 200 µL.
6. Press START on the keypad and position the plates according to the instructions in the display window.
7. Place the 96 Standard Plate (containing the 96 Deep Well Tip Comb) in the Loading Position. Press START to move the turntable for the placement of the next plate.
8. Place the Elution plate loaded with Elution Buffer (see Step 5) in the loading position; press START to move the turntable.
9. Place the Wash 2 plate loaded with Wash Buffer (see Step 4) in the loading position; press START to move the turntable.
10. Place the Wash 1 plate loaded with Wash Buffer (see Step 4) in the loading position; press START to move the turntable.

Automated Purification of DNA from the Samples

1. Preparing the Samples

a. Aliquot 100 µL of each test sample to individual wells of a 96 Deep Well Plate, following the plate layout you specified.

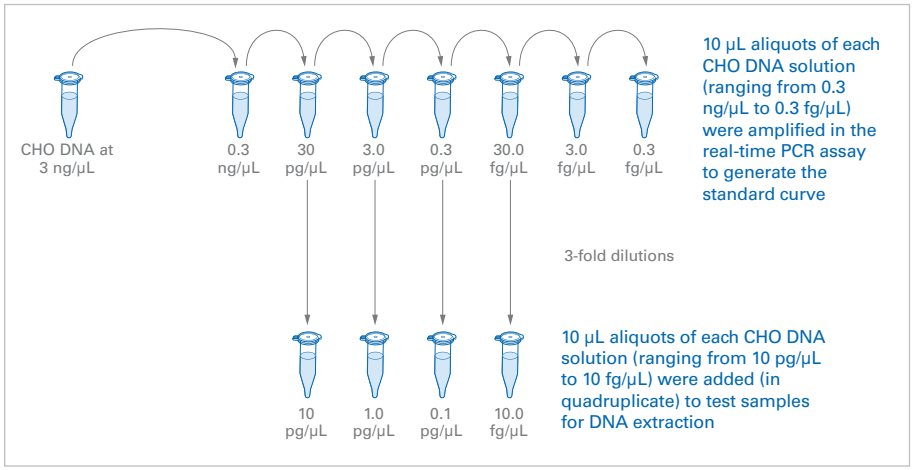

Figure 2. Preparation of DNA Dilutions for Standard Curve and Spike.

Note: To accommodate the additions of proteinase K and proteinase K Buffer, the maximum sample volume that can be added to each well of the plate is 100 µL.

b. Adjust pH, if necessary.

Note: Check pH of the samples with pH paper. The DNA recovery can be maximized by adjusting the pH to between 6 and 8 using 10 N NaOH or HCl.

c. Add 60 µL of proteinase K Buffer and 10 µL of proteinase K.

d. Add 10 µL of 5 M NaCl if the salt concentration of the sample is lower than 0.5 M.

Note: The DNA recovery is maximized if the NaCl concentration is ≥0.5 M. Whether or not the starting NaCl concentration is known, it is generally acceptable to add 1/10 volume of 5 M NaCl to ensure the sample is at least 0.5 M NaCl. It is important to adjust pH first before adding more NaCl. Adding concentrated NaCl to low-pH solutions can cause protein precipitates that make sample preparation more difficult.

e. Centrifuge the plate at 1000 rpm for 1 minute to collect the contents of the wells to the bottom.

f. Place the plate in the instrument in the loading position. Press START. Mix for 10 seconds at fast speed; incubate at 56˚C for 30 minutes with mixing at slow speed.

2. Adding Lysis Solution, Magnetic Particles, and DNA Binding Solution

a. At the end of the proteinase K incubation step, the Lysis plate will be in the loading position, and the instrument will be on PAUSE.

b. Remove the plate from the instrument. Add 360 µL of Lysis Solution using an 8-channel pipette. The Lysis Solution should be freshly supplemented with glycogen and yeast tRNA at final concentrations of 8 µg/µL and 10 µg/µL, respectively. Pipette up and down two times to mix.

c. Vortex the bottle containing the magnetic particles until the particles are completely resuspended.

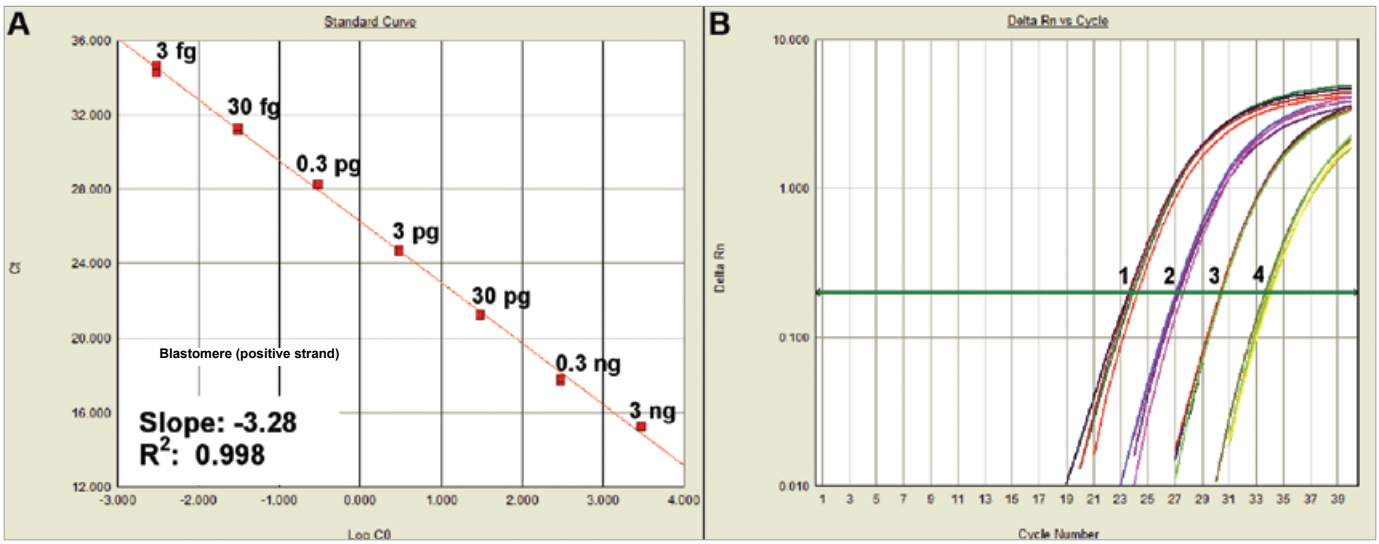

Figure 3. A. Standard Curve Generated by the Amplification of Serially Diluted CHO DNA. **B.** Amplification Plot of CHO DNA Test Samples Extracted Using the MagMAX™ Express 96 Processor and the PrepSEQ™ Residual DNA Sample Preparation Kit (1 = 100 pg spike; 2 = 10 pg spike; 3 = 1 pg spike; 4 = 0.1 pg spike).

d. Add 30 µL of magnetic particle suspension to the sample. Shake the plate gently to mix. Avoid spilling sample from one well to the next while mixing.

e. Add 300 µL of Binding Solution using an 8-channel pipette. Pipette up and down two times to mix.

f. Place the plate back to the Loading Position in the instrument. Press START to move to the binding step. Mix the beads for 15 minutes at superfast speed.

g. Collect beads with 45 counts.

3. Washing and Eluting

a. Washing and eluting are performed automatically in the instrument. Wash 1 and 2 steps take 1 minute each with medium-speed mixing. Collect beads with 15 counts.

b. Air dry the beads for 15 seconds.

c. Elute DNA at 70˚C for 5 minutes with fast mixing.

d. Collect beads with 15 counts.

e. The comb with beads will be washed in the Wash 2 plate and placed on the comb loading plate.

f. The Elution plate will be positioned in the loading position at the end of the procedure. The eluates are ready for analysis.

4. Measuring the Volume of the Eluates

In order to calculate the recovery rate in the spiked samples, it is necessary to measure the final volume of the eluates, since volume loss (through evaporation) can occur during the 70˚C incubation period.

The eluate volume can be measured using a pipette. We suggest that 3 to 5 wells of the Elution plate that were not used for the sample prep be preloaded with either 100 or 200 µL of Elution Buffer (depending on the volume in which you chose to elute your samples). At the end of the procedure, measure the volume of those wells using a pipette, calculate an eluate volume average, and then apply that average volume to all samples during the recovery rate calculation.

Quantifying Extracted DNA

Real-time PCR reactions were carried out using 10 µL of each extracted DNA, and components of the resDNASEQ™ Quantitative CHO DNA Kit. A real-time PCR standard curve was generated by the amplification of 10 µL of each of the CHO DNA standard curve samples, added in the following amounts: 3.0 ng, 0.3 ng, 30.0 pg, 3.0 pg , 0.3 pg, 30 fg, and 3.0 fg.

TABLE 1. RECOVERY RATE FROM SAMPLES WHERE CHO DNA WAS ADDED TO IGG-CONTAINING TEST SAMPLES AND PURIFIED USING THE AUTOMATED PROCEDURE.

Amount of Spiked DNA (pg)	Amount of Recovered DNA (pg)			Average Recovery	%CV
100	105	133	123	120%	12%
10	10.2	10.9	11.5	109%	6%
1	1.1	1.1	1.2	110%	5%
0.1	0.09	0.1	0.08	90%	7.90%

Results

Figure 3 shows (A) the standard curve generated by the amplification of CHO DNA, and (B) the amplification plot of the test samples containing spiked CHO DNA, that were purified using the automated procedure. The standard curve gave a slope of –3.28, demonstrating the high efficiency of the PCR reaction, and an R^2 value of 0.998, showing excellent linearity. The amount of DNA in each spiked sample well was calculated by comparison with the standard curve.

Table 1 shows the recovery of CHO DNA from samples where 100 pg, 10 pg, 1 pg, and 0.1 pg of CHO DNA were added. These data show that the DNA was recovered with very high efficiency using the automated procedure, with sample replicates ranging from 90% to 120% recovery. Note that only one PCR reaction was performed for each extracted DNA. The standard deviation can potentially be improved by running replicate PCR reactions for each extracted sample.

Conclusions

The automatic sample preparation procedure can effectively extract DNA from a solution containing 100 mg/mL IgG. This high recovery rate is consistent when spiked DNA amounts from 0.1 pg to 100 pg are added. The procedure can process samples in a 96-well plate format in 2 hours and can extract small amounts of DNA from complex mixtures of proteins, buffers, and salts, which are typical of samples that need to be quantitated for residual host cell line DNA. Additional experiments have produced similar performance with other sample types; high recovery rates were observed in all wells.

ORDERING INFORMATION

Description	P/N
resDNASEQ™ Quantitative CHO DNA Kit + PrepSEQ™ Residual DNA Sample Preparation Kit 100 rxn, without Protocol and Quick Reference Card	4413713
resDNASEQ™ Quantitative CHO DNA Kit 100 rxn, without Protocol and Quick Reference Card	4402085
PrepSEQ™ Residual DNA Sample Preparation Kit 100 rxn, without Protocol and Quick Reference Card	4413686
resDNASEQ™ CHO Genomic DNA Standard 100 rxn	4403965
MagMAX™ Express 96 DW, Applied Markets	4400079
MagMAX™ Express-96 Standard Plates	4388475
MagMAX™ Express-96 Deep Well Tip Combs	4388487
MagMAX™ Express-96 Deep Well Plates	4388476

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