Promega

Product Application

High-Throughput Plasmid Purification with Large-Volume Bacterial Cultures

Automated plasmid DNA extraction from 30ml of bacterial culture using a modified version of the Wizard MagneSil Tfx™ System protocol on the Tecan Freedom EVO® 200 instrument. This protocol was developed in partnership with Innate Pharma, a French biotechnology company developing first-in-class therapeutic antibodies to improve cancer treatment.

Kit: Wizard MagneSil Tfx™ System (Cat.# A2380)

Analyses: Plasmid quantification, cell transfection for antibody production

Sample Type(s): Bacterial culture of *E. coli* strain transformed with plasmids encoding antibody light or heavy chains (Transformed bacteria were cultured in Luria Bertani or Terrific Broth medium for 16 hours.)

Input: Cell pellet from 30ml of bacterial culture

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Bulletin #TB314, available at: www.promega.com/protocols

or e-mail Technical Services at: techserv@promega.com

Materials Required:

- Tecan Freedom EVO® 200 Liquid Handler
- Bioshake D30 t-elm and adapter plate (QInstruments)
- Alpaqua MagPlate 24 (Cat.# A000270)
- Triton® X-100 (Sigma-Aldrich X100-100ml)
- isopropanol and absolute ethanol (molecular biology grade)
- 24-well deep-well plates (Whatman UniPlates™ Cat.# 7701-5102)
- 1.5ml microtubes

Protocol:

The procedure described below is a modification of the protocol in the *Wizard MagneSil Tfx™ System Technical Bulletin* #TB314 adapted for automation on the Tecan Freedom EVO® 200 in a 24-well plate format.

1. Add Triton® X-100 to the Cell Lysis Buffer to a final concentration of 1%.
   Note: This step is a modification of the standard protocol in #TB314.
2. Prepare 80% ethanol.
3. If not already in the appropriate 24-well deep-well plate, manually resuspend bacterial pellets with 900µl of Cell Resuspension Solution by vortexing and tip-mixing.
4. Transfer to an empty 24-well deep-well plate.
5. The method uses the reagents and volumes per well as listed in Table 1.
   Note: Isopropanol addition during the binding step is a modification of the standard protocol in #TB314.

Table 1. Reagents and Volumes Used for the Automated Wizard MagneSil Tfx™ System Protocol.

Reagents	Volume Used per Well (µl)
Cell Resuspension Solution	900
Cell Lysis Solution **(with 1% Triton® X-100)**	1200
Neutralization Solution	1200
MagneSil® BLUE	300
Endotoxin Removal Resin or Beads	250
MagneSil® RED	400
isopropanol	2000
4/40 Wash Solution	2000
80% ethanol	2 × 1900
Elution Buffer (Nuclease-Free Water)	500

6. Start the run on the Tecan Freedom EVO® 200 liquid handler.
7. Summary of main steps of the EVOware® script:
   - Lyse bacteria with Cell Lysis Solution (containing 1% Triton® X-100).
   - Neutralize lysis with Neutralization Solution.
   - Capture cell debris with MagneSil® BLUE resin.
   - Transfer cleared lysates to a new deep-well plate.
   - Treat lysate with Endotoxin Removal Resin.
   - Bind plasmid with isopropanol and MagneSil® RED resin in a new deep-well plate.
   - Wash resin once with 4/40 Wash Solution and twice with 80% ethanol.
   - Dry resin.
   - Elute DNA with Elution Buffer (Nuclease-Free Water).

   Note: The elution volume is set up by the user during the run preparation.

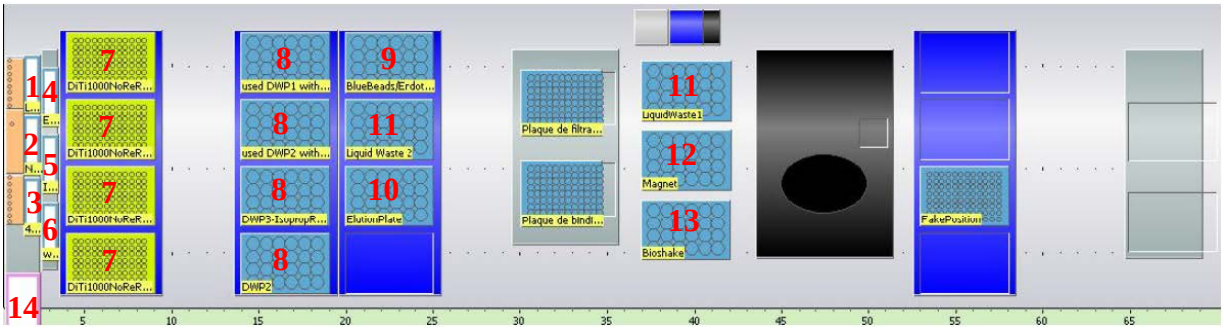

Figure 1. Tecan Freedom® EVO 200 deck layout. Labels of used positions on the deck:

1. Cell Lysis Solution (with 1% Triton® X-100)
2. Neutralization Solution
3. 4/40 Wash Solution
4. 80% ethanol
5. Isopropanol
6. Liquid waste
7. Tips racks
8. 24-well deep-well plate
9. Plate containing MagneSil® BLUE, Endotoxin Removal Resin, MagneSil® RED and Elution Buffer for distribution
10. Elution plate
11. Liquid waste
12. Magnet
13. Heater/shaker
14. Tip waste

Results:

Plasmid DNA encoding for antibody light and heavy chains (clone H and K) were purified from 30ml of overnight bacterial cultures, grown in Luria Bertani or Terrific Broth culture media, using the automated Wizard MagneSil Tfx™ System. Following purification, yield and purity were measured by absorbance (Figure 2). Cells were transfected with the purified plasmids and incubated for 6 days to produce antibodies. Antibodies were purified using affinity chromatography and IgG quantified using a NanoDrop™ Spectrophotometer (Figure 3). Endotoxin levels of the purified plasmids also were measured (Figure 3).

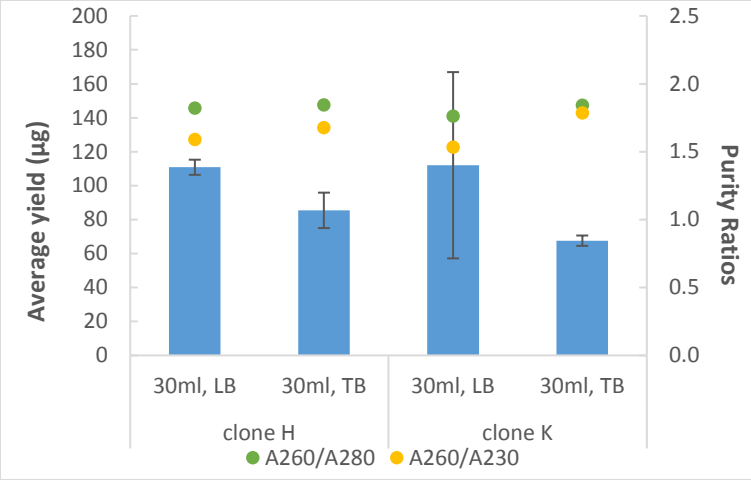

Figure 2. Average yield and purity of plasmid DNA by automated purification. Yield and purity of plasmid DNA purified using the automated Wizard MagneSil Tfx™ System were measured by absorbance on a NanoDrop™ Spectrophotometer. Shown is the average yield ± standard deviation for three replicates. Clone H is a proprietary plasmid encoding the antibody heavy chain; clone K is a proprietary plasmid encoding the antibody light chain.

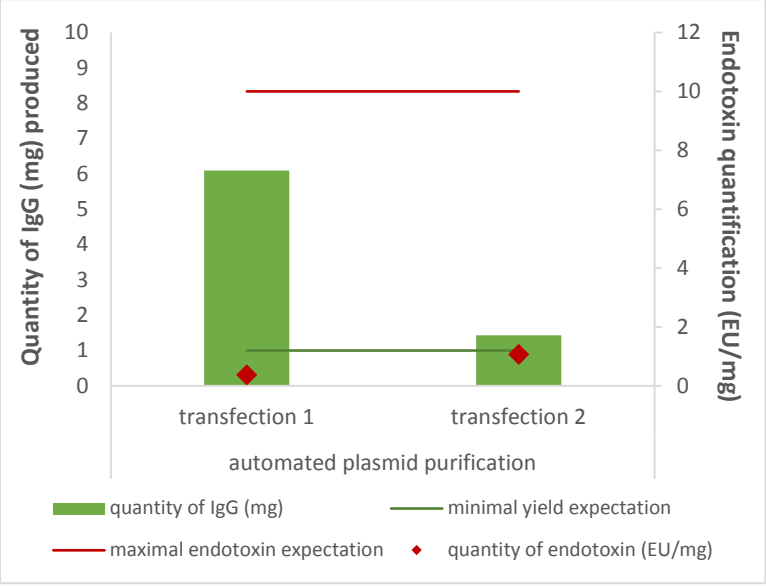

Figure 3. Quantification of antibody production and endotoxin levels. Results from cell transfections with plasmids purified using the automated Wizard MagneSil Tfx™ System.

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