

High-Throughput Plasmid Purification with Large-Volume Bacterial Cultures on the Hamilton STAR Liquid Handler

Automated plasmid DNA extraction from up to 25ml of bacterial culture using a modified version of the Wizard MagneSil Tfx™ System protocol on the Hamilton STAR liquid handler.

Kit:	Wizard MagneSil Tfx™ System (Cat. # A2380)
Analyses:	UV absorbance (NanoDrop™)
Sample Type(s):	Bacterial cell pellets for plasmid isolation (cultured in Luria Bertani medium for at least 18 hours)
Input:	Up to 200mg of bacterial biomass (equivalent to ~25ml of culture at OD ₆₀₀ = 2.0)
Materials Required:	<ul style="list-style-type: none">▪ Hamilton STAR Liquid Handler▪ Hamilton Heater Shaker (HHS) Device▪ Alpaqua MagPlate 24 (Cat.# A000270)▪ Isopropanol and absolute ethanol (molecular biology grade)▪ 24-well deep-well plates (Whatman UniPlates™ Cat.# 7701-5502)▪ 15ml conical tubes▪ Bio-Rad Hard-Shell® 96-Well PCR Plate (Bio-Rad, Cat.# HSP9601)

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.

Further information can be found in Technical Bulletin #TB314, available at: www.promega.com/protocols

or by e-mailing technical services at: techserv@promega.com

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 Hamilton STAR Liquid Handler in a 24-well plate format. Once the re-suspended bacterial pellet is placed on the liquid handler deck, the remainder of the process is fully automated.

1. Prepare 80% ethanol.
2. Manually resuspend bacterial pellets with 900µl of Cell Resuspension Solution by vortexing and tip-mixing and add each sample to a well of an empty 24-well deep-well plate.
3. The method uses the reagents and volumes per well as listed in Table 1.

Table 1. Reagent volumes required per sample processed on the Wizard MagneSil Tfx™ method for the Hamilton STAR Liquid Handler

Reagent	Volume
Cell Resuspension Solution (CRA)	900µl
Cell Lysis Solution (CLA)	1.2ml
Neutralization Solution (NSA)	1.25ml
MagneSil® BLUE	500µl
Endotoxin Removal Resin	250µl
MagneSil® RED	400µl
Isopropanol	3500µl
4/40 Wash Solution	1ml
Ethanol	1.9ml
Nuclease-free Water	30-1000µl (user selected volume)

4. Note: Isopropanol addition during the binding step is a modification of the standard protocol in #TB314.
5. Start the run on the Hamilton STAR Liquid Handler.
6. Summary of the main steps of the Hamilton STAR script:
 - Lyse bacteria with Cell Lysis Solution.
 - 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 with 80% ethanol.
 - Dry resin.
 - Elute DNA with Elution Buffer (Nuclease-Free Water).

Note: The elution volume is selected by the user during the run setup.

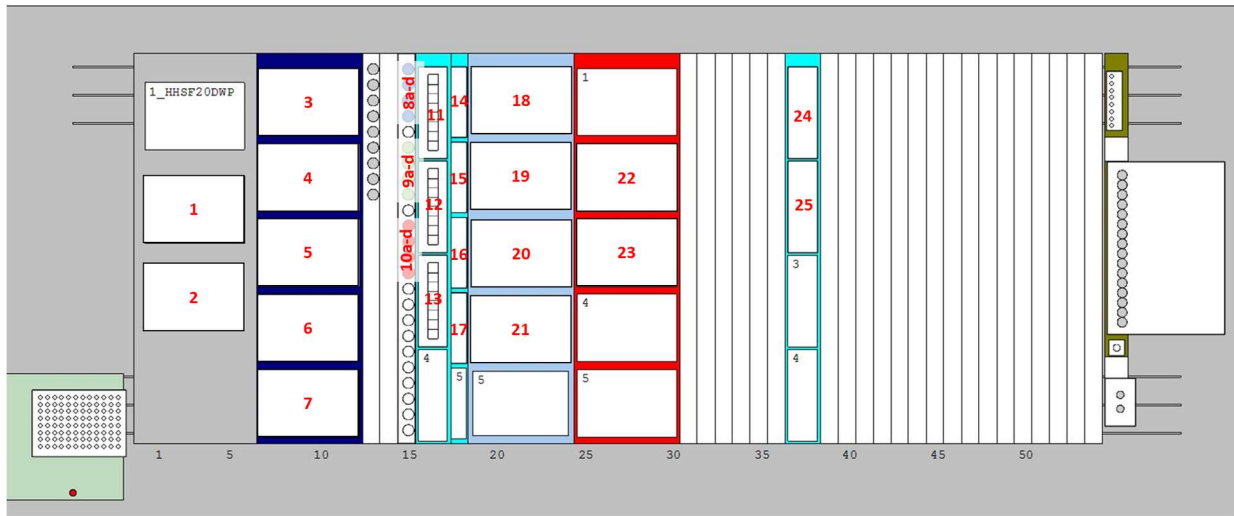


Figure 1. Hamilton STAR deck layout for processing samples with the Wizard MagneSil Tfx™ System.

1. 24-well deep-well plate with resuspended bacterial cell pellets (on HHS; for 1-24 samples).
2. 24-well deep-well plate with resuspended bacterial cell pellets (on HHS; only if processing 25-48 samples).
3. Processing plate 24-well deep well plate (for 1-24 samples).
4. Processing plate 24-well deep well plate (only if processing 25-48 samples).
5. Alpaqua Ring Magnet (for 1-24 samples).
6. Alpaqua Ring Magnet (for 25-48 samples).
7. Elution Plate (Bio-Rad PCR Plate).
8. 15ml conical tube with MagneSil® BLUE Resin (In tube positions 1-4)
9. 15ml conical tube with Endotoxin Removal Resin (In tube positions 6-9)
10. 15ml conical tube with MagneSil® RED Resin (In tube positions 11-14)
11. Empty 200ml Reservoir for Waste
12. Empty 200ml Reservoir for Waste
13. Empty 200ml Reservoir for Waste
14. 4/40 Wash in 50ml Reservoir
15. Cell Lysis Solution (CLA) in 50ml Reservoir
16. Neutralization Solution in 50ml Reservoir
17. Elution Buffer (Nuclease-free Water) in 50ml Reservoir
18. 1ml Filtered Tips (full rack)
19. 1ml Filtered Tips (full rack)
20. 1ml Filtered Tips (full rack)
21. 300µl Filtered Tips (full rack)
22. Processing plate 24-well deep-well plate (for 1-24 samples)
23. Processing plate 24-well deep-well plate (only if processing 25-48 samples).
24. 80% Ethanol in 200ml Reservoir
25. Isopropanol in 200ml Reservoir

Results:

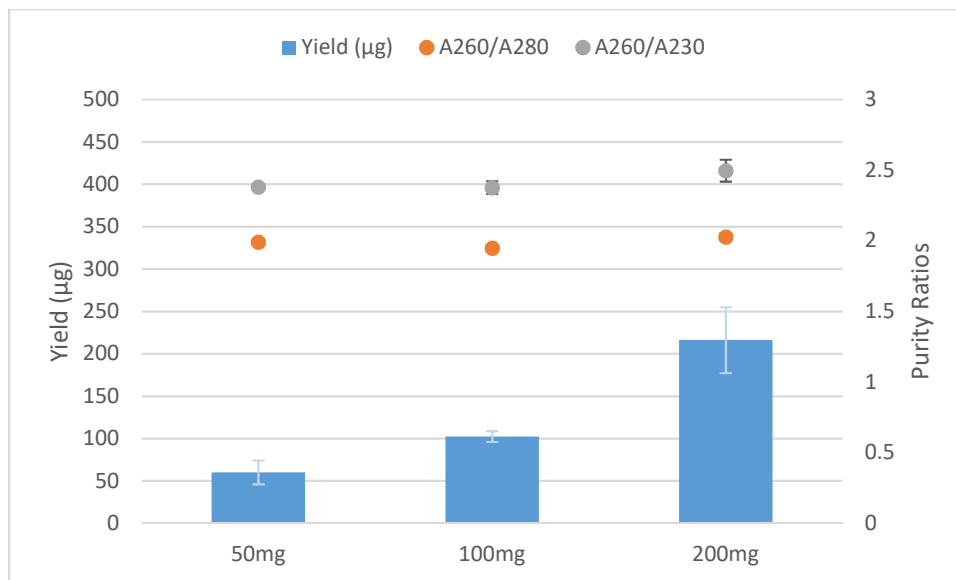


Figure 2. Purification of plasmid DNA from up to 200mg of bacterial cell biomass with the Wizard MagneSil Tfx™ System on the Hamilton STAR Liquid Handler. Yields were determined by UV absorbance at 260nm on the NanoDrop™ 8000 Instrument and were linear with up to 200mg of bacterial biomass input. Purity ratios were also determined based on UV absorbance. Shown is mean ± standard deviation (n=3).