

DNA Purification from Gram-Negative Bacteria

Purify DNA from a direct culture or cell pellet of gram-negative bacteria using the Maxwell® RSC Whole Blood DNA Kit.

Kit: Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520)

Analysis: Nanovue®, Quantus™ Fluorometer and qPCR

Sample Type(s): *Escherichia coli* in liquid culture

Input: up to 10⁹ bacteria

Materials Required:

- Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Optional: Tissue Lysis Buffer (TLA) (Cat.# A5091)

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

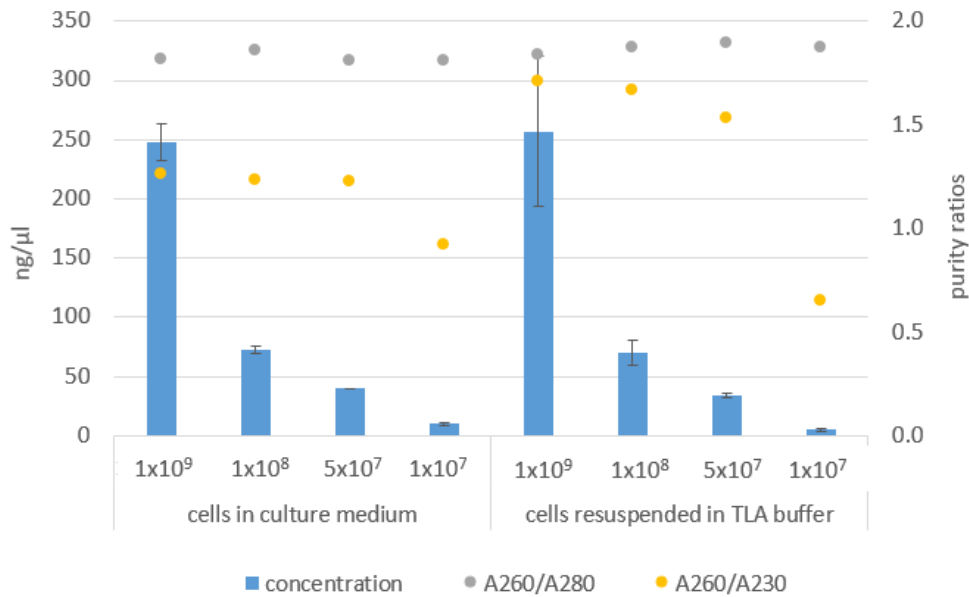
Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM455, available at: www.promega.com/protocols, or e-mail: techserv@promega.com

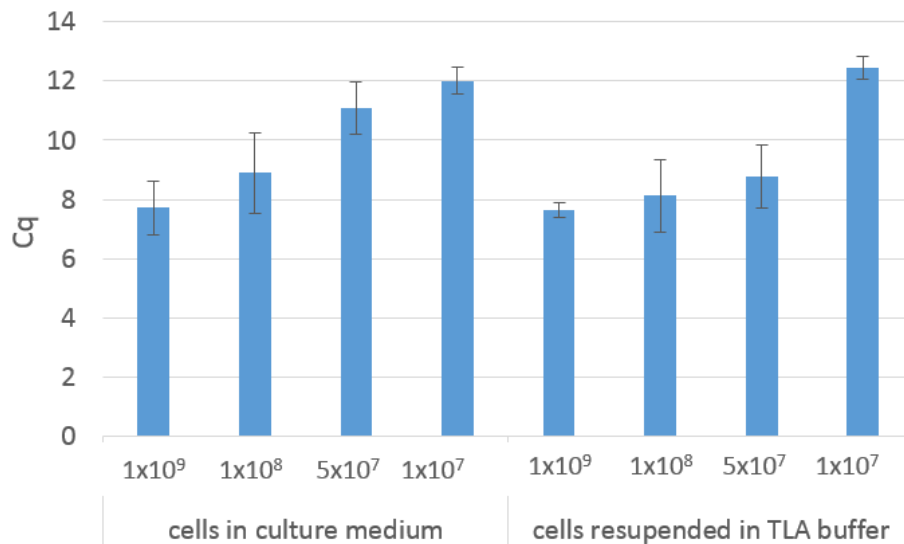
Protocol:

1. Add 400µl of *E. coli* culture in culture medium to well 1 of a RSC Whole Blood cartridge.
Optional: For better purity, the culture can be centrifuged for 2 minutes at 13000rpm and the bacterial pellet resuspended in 400µl of Tissue Lysis Buffer (TLA) before addition to well 1.
2. Add a plunger to well 8 of the cartridge.
3. Place an elution tube into the elution tube position and add 60µl of elution buffer.
4. Run the Whole Blood protocol on the Maxwell® RSC Instrument.

Results:



DNA concentration and purity ratios. DNA was purified from 1×10^9 , 1×10^8 , 5×10^7 and 1×10^7 bacteria using the Maxwell[®] RSC Whole Blood DNA Kit with or without the Tissue Lysis Buffer (TLA) resuspension option. DNA concentration was measured using NanoVue[®], Quantus[™] Fluorometer or absorbance ratios.



qPCR amplification. Cq values obtained for 1ng of DNA extracted from *E. coli* amplified by qPCR using 16S rRNA primers (1).

Reference:

1. Brow, M.A. *et al.* (1996) Differentiation of bacterial 16S rRNA genes and intergenic regions and *Mycobacterium tuberculosis* katG genes by structure-specific endonuclease cleavage. *J. Clin. Microbiol.* **34**, 3129–37.