Promega

Product Application

Automated Purification of Host Cell DNA from Biologics

This protocol is a suggested approach for purifying E. coli host cell DNA from antibody biologics using the Maxwell® RSC Instrument and the Maxwell® RSC Cell DNA Purification Kit. The assay has not been fully optimized.

Kit: Maxwell® RSC Cell DNA Purification Kit (Cat.# AS1370)

Analyses: qPCR

Samples: The following buffers were tested in phase 1 development to include a high and low salt option, both Tween®-20 and Triton® X-100 detergents, and an acidic elution buffer neutralized with Tris. Buffers were prepared in a bacteria-free hood with fresh reagents to minimize bacterial DNA contamination. Hot Start Antibody 7 was added at 5mg/ml to mimic an antibody-based biologic. *E. coli* Genomic DNA was spiked into an aliquot of each buffer at a final concentration of 0pg/100µl (unspiked) or 100pg/100µl.

- Buffer A: 50mM Tris, pH 8.0, 0.1mM EDTA, 100mM NaCl, 5mg/ml Hot Start Antibody 7
- Buffer B: 50mM Tris, pH 8.0, 0.1mM EDTA, 100mM NaCl, 0.1% Tween®-20, 5mg/ml Hot Start Antibody 7
- Buffer C: 50mM Tris, pH 8.0, 0.1mM EDTA, 100mM NaCl, 0.1% Triton® X-100, 5mg/ml Hot Start Antibody 7
- Buffer D: 0.1M glycine, pH 2.5 neutralized with 2/5 volume 2M Tris, pH 8.0, 5mg/ml Hot Start Antibody 7
- Buffer E: 50mM NaH_2PO_4, 1M NaCl, pH 7.2, 5 mg/ml Hot Start Antibody 7

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 Manual TM418, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Input: 100µl sample, undiluted

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Cell DNA Purification Kit (Cat.# AS1370)

Protocol:

1. Prepare up to 16 Maxwell® RSC Cell DNA cartridges and elution tubes as indicated in the Maxwell® RSC Cell DNA Purification Kit Technical Manual (TM418), taking care to minimize bacterial contamination.
2. Add 100µl of undiluted or neutralized sample directly to well #1 of the Maxwell® RSC cartridge. Pre-processing is not required.
3. Purify DNA on the Maxwell® RSC Instrument using the Cell DNA method.

Results:
Several Maxwell® RSC purification chemistries were initially surveyed across the 5 test buffers. The Maxwell® RSC Cell DNA Purification Kit was selected due to the combination of low background bacterial DNA in purification blanks and relatively high DNA recovery from 500pg of spiked *E. coli* genomic DNA (63% across all buffers after subtraction of background DNA sources, data not shown).

Testing was repeated, purifying DNA with the Maxwell® RSC Cell DNA Purification Kit in triplicate from 100µl of each sample buffer spiked with 0pg or 100pg of *E. coli* DNA per 100µl sample (Figure 1). Recovery averaged 65% across all buffers (range 46% to 74%), similar to the first round of testing. Purification appears robust across two kit lots, low and high salt buffers, as well as detergents in this minimal sample set.

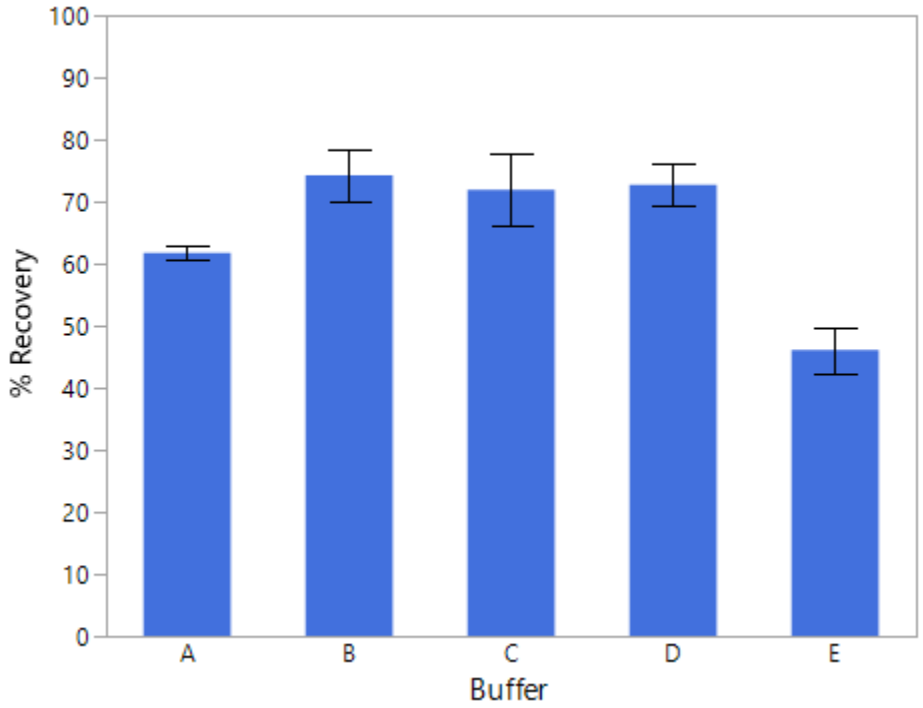

Figure 1. Recovery of 100pg *E. coli* DNA per 100µl sample buffer using the Maxwell® RSC Cell DNA Purification Kit on the Maxwell® RSC Instrument. Buffers (including 5mg/ml antibody) were spiked with either 0pg or 100pg of *E. coli* genomic DNA per 100µl and purified in triplicate using the Maxwell® RSC Cell DNA Purification Kit. Total bacterial DNA was quantified in duplicate from 5µl of each sample using an in-house qPCR-based assay, including a standard curve of *E. coli* genomic DNA down to 40 copies of the qPCR target, and 6 no-template control reactions. Recovery was calculated by subtracting the background DNA recovered from unspiked samples (0pg), adjusting for average eluate volume (44µl) and qPCR input (5µl), and dividing by the number of target copies expected in the 100pg spike (15,718 copies). Mean ± standard deviation is shown for duplicate amplifications of triplicate purifications per buffer.