Promega

# Product Application

## Automated Purification of DNA from Human Bone Marrow Aspirate

*Purify DNA from cell subpopulations in bone marrow aspirates using the Maxwell® RSC Instrument and Maxwell® RSC Buffy Coat DNA Kit.*

**Kit:** Maxwell® RSC Buffy Coat DNA Kit (Cat.# AS1540)

**Analyses:** UV absorbance and ddPCR

**Sample Type(s):** Human Bone Marrow Aspirate spiked with RAW264.7 mouse cells

**Input:** 300µl

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 TM464 **www.promega.com/protocols**

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

**Materials Required:**

- Bone Marrow Aspirate (Lonza, Cat.# 1M-125)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Buffy Coat DNA Kit (Cat.# AS1540)
- NanoDrop™ Spectrophotometer
- Bio-Rad CFX96 Real-Time PCR Detection System
- Droplet Generation Oil for Probes (Bio-Rad, Cat.# 1863005)
- DG8 Cartridges (Bio-Rad, Cat.# 1864008)
- Droplet Generator DG8 Gasket (Bio-Rad, Cat.# 1863009)
- Pierceable Foil Heat Seal (Bio-Rad, Cat.# 1814040)
- ddPCR Supermix for Probes (no dUTP) (Bio-Rad, Cat.# 1863024)
- Bio-Rad QX200 Droplet Generator
- Bio-Rad C1000 Touch Thermal Cycler
- Bio-Rad PX1 Plate Sealer
- Bio-Rad QX200 Droplet Reader
- Primers and Probe from IDT
  - Mouse Forward 5'-TAC ACC CTG AGC AAT GTC CA-3'
  - Mouse Reverse 5'-CTG TGG CAG GCA ATT AGG AT-3'
  - Mouse Probe 5'-/56-FAM/ACT CAC CAG /ZEN/GCA CTA TCA GAT AGA GTC C/3IABkFQ/-3'

**Protocol**:

1. Samples were prepared by adding 10µl of a RAW264.7 cell suspension to 990µl of bone marrow. The samples were prepared in order to have 10, 100, and 1,000 cells/cartridge.
2. Add 300µl of prepared bone marrow directly into well #1 of the Maxwell® RSC Buffy Coat DNA cartridge and mix by pipette at least 10 times.
3. Complete purifications following the Maxwell® RSC Buffy Coat DNA Kit Technical Manual (TM464) and eluting in the recommended 200µl Elution Buffer.

**Results:**

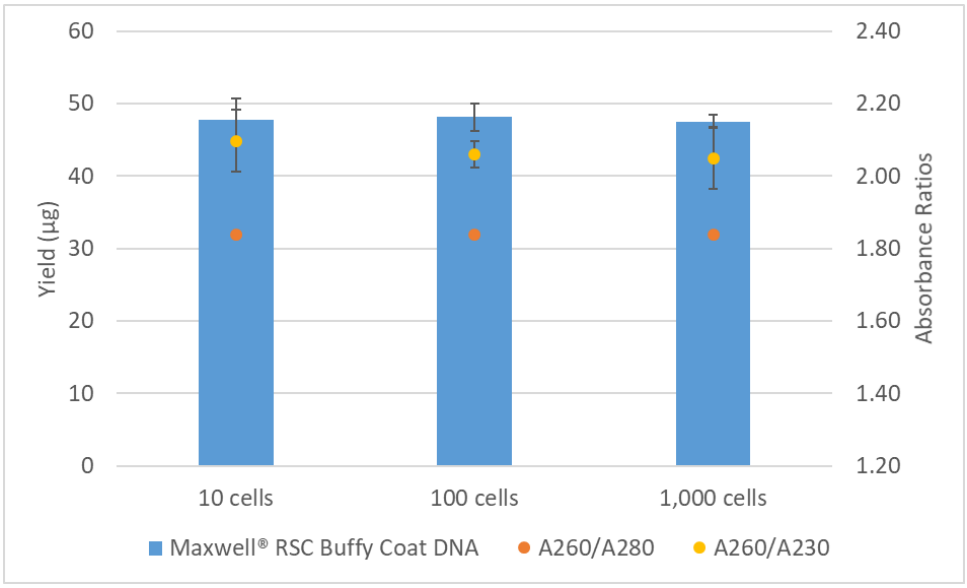


**Figure 1. Yield and purity ratios for DNA purification from bone marrow aspirate.** DNA was purified using the Maxwell® RSC Buffy Coat DNA Kit on the Maxwell® RSC Instrument. Concentration of purified DNA was quantified using absorbance (NanoDrop™, Thermo Fisher Scientific). Yields were calculated from elution volume. Purity ratios were measured by absorbance (NanoDrop™). Data represent the Mean ± STD of N=3.

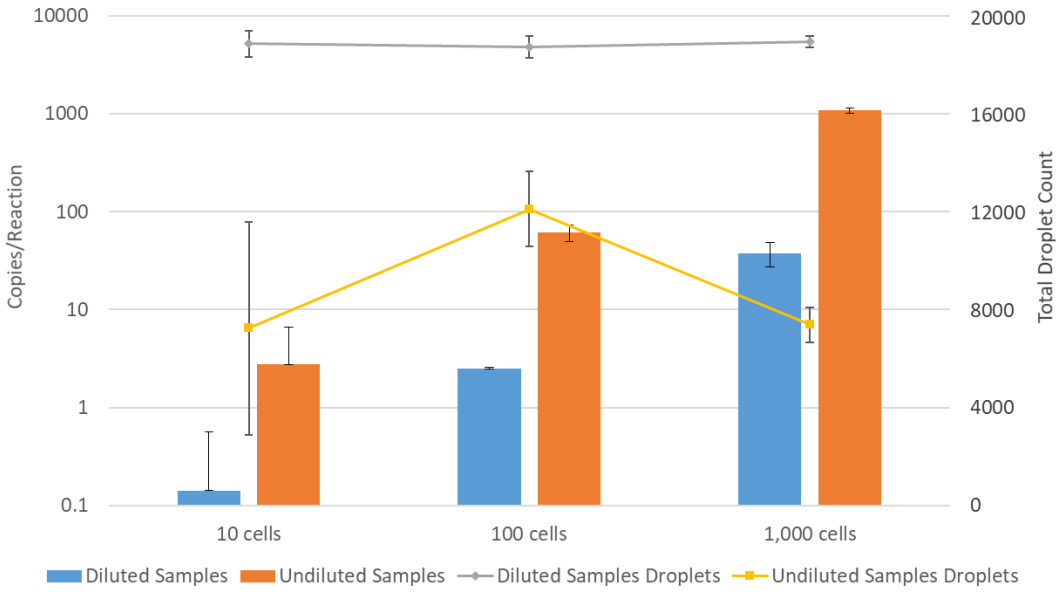


**Figure 2. Digital Droplet PCR (ddPCR) sample analysis.** 5µl of sample diluted to 10ng/µl and 5µl of undiluted sample were analyzed in a mouse specific ddPCR workflow. In all cases, the 100 cell samples consistently gave positive droplets, whereas the 10 cell samples did not. The addition of more sample DNA increased the amount of 10 cell samples with positive droplets but decreased overall droplet formation which indicates a possible decline in assay performance.