Promega

Product Application

Droplet Digital PCR Compatibility with Maxwell® RSC Cultured Cells DNA Kit Purification

DNA was purified from a suspension of HCT116 cells using the Maxwell® RSC Cultured Cells DNA Kit and quantitated via Droplet Digital PCR.

Kit: Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620)

Analyses: Bio-Rad ddPCR™ Copy Number Variation Assays, Validated

Sample Type: HCT116 cells in McCoy's Media +10% FBS

Input: 10^6 cells in 400µl of McCoy's Media

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 #TM477, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620)
- Bio-Rad ddPCR™ Copy Number Variation Assays, Validated

Protocol:

1. Purify DNA from cultured cells using standard protocol outlined in technical manual #TM477.
2. Dilute DNA to a maximum of 10ng/µl based on QuantiFluor® ONE dsDNA System (Cat.# E4870).
3. Set up ddPCR amplification reactions using the Bio-Rad ddPCR™ Copy Number Variation Assays, Validated protocol (10033173 Ver C), with a maximum of 50ng DNA input. Do not digest DNA prior to the reactions and do not use restriction enzymes in the reactions.

Results:

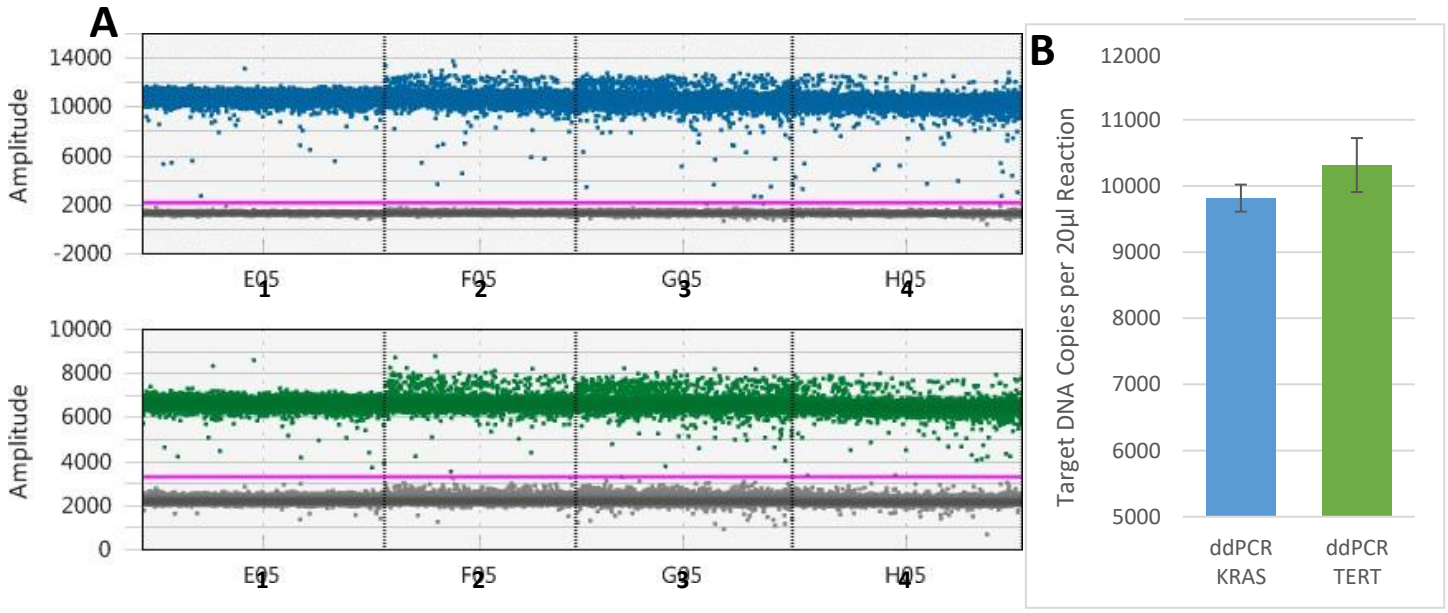

Figure 1. Droplet digital PCR quantitation of DNA purified from HCT116 cell cultures.
DNA was purified from 10^6 HCT116 cells suspended in 400µl of McCoy's media using the Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620) and diluted to 10ng/µl based on QuantiFluor® ONE dsDNA System (Cat.# E4870). Amplification reaction mixtures were set up based on the Bio-Rad ddPCR™ Copy Number Variation Assays, Validated protocol, with CNV assays for human KRAS (FAM) and TERT (HEX), using 50ng of DNA input. Panel A (left) displays the 1D droplet plot for ddPCR amplification of KRAS (blue) and TERT (green) targets. The threshold, above which droplets are considered positives (contain target DNA), is shown in pink. Each column (labeled 1-4 beneath each plot) represents a different sample replicate. The Y-axis is relative fluorescence. Droplets with intermediate fluorescence (rain) indicate inefficiently amplified target DNA. Panel B (right) displays the average copies of KRAS and TERT DNA targets per ddPCR reaction calculated using QuantaSoft™ Analysis Pro Software.