Promega

Product Application

Automated Purification of RNA from Whole Blood for BCR-ABL Detection

Purify RNA of sufficient quality and quantity for detection of BCR-ABL transcripts using the Maxwell® RSC Instrument and the Maxwell® RSC simplyRNA Blood Kit for end-point PCR, RT-qPCR, and ddPCR applications.

Kit: Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)

Analyses: End-point PCR, RT-qPCR, and ddPCR

Sample Type(s): RNA from K562 cells spiked into 2.5ml of whole blood

Materials Required:

- Maxwell® RSC simplyRNA Blood Kit (Cat. #AS1380)
- Maxwell® RSC Instrument (Cat.# AS4500)

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 TM417 and TM337, available at: **www.promega.com/protocols**

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

Protocol: **RNA Purification**

1. Transfer 2.5ml of well mixed, fresh whole blood from the EDTA collection tube into a 15ml tube.
2. Add 7.5ml of Cell Lysis Solution and invert the tube 5-6 times to mix.
3. Incubate lysates for 10 minutes at room temperature. Twice during the incubation, invert to mix.
4. Centrifuge tube at 3,000 x *g* for 10 minutes.
5. Remove and discard as much of the supernatant as possible without disturbing the visible white pellet. Briefly spin to collect residual liquid at the bottom of the tube and remove and discard the supernatant with a pipette.
   Optional: As a positive control, add 100µl of K562 cells to white blood cell pellets. Centrifuge tube again at 3,000 x g for 10 minutes.
6. Proceed with the standard protocol for sample, Maxwell® RSC Cartridge, and Maxwell® RSC Instrument preparation according to the Maxwell® RSC simplyRNA Blood Kit Technical Manual (TM417).

Protocol: GoTaq® 2-Step RT-qPCR System (Cat.# A6010)

1. Combine 900ng of RNA with 1µl each of Random Primer and Oligo(dT)$_{15}$ Primer.
2. Denature the RNA and primers at 70°C for 5 minutes. Chill at 4°C for 5 minutes.
3. Prepare the GoScript™ Reaction Mix and combine with the denatured RNA and primers.
4. Synthesize cDNA using the GoScript™ RT, dilute cDNAs 1:4 in Nuclease-Free Water and amplify using GoTaq® qPCR Master Mix, according to the GoTaq® 2-Step RT-qPCR System Technical Manual (TM337).

Protocol: ddPCR EvaGreen Supermix (BioRad, Cat.# 1864034)

1. Synthesize cDNA using GoScript™ Reverse Transcriptase as described above.
2. Add 5µl of the diluted cDNAs to a 20µl EvaGreen Supermix reaction.
3. Create droplets on the QX200 Droplet Generator, amplify cDNA using the Biorad C1000 Touch Thermal Cycler, and detect using the QX200 Droplet Reader.

Protocol: cDNA Amplification using the Invivoscribe BCR/ABL t(9;22) Translocation Assay (Invivoscribe Cat.# 13100010)

1. Generate cDNAs as described above using GoScript™ Reverse Transcriptase.
2. Add 5µl of the diluted cDNAs from test and control samples into each reaction master mix.
3. Amplify cDNAs using AmpliTaq™ Gold DNA Polymerase and a recommended thermocycler according to the Technical Manual.
4. Run 20µl of each PCR reaction on a 2% agarose gel for detection of amplified products.

Results

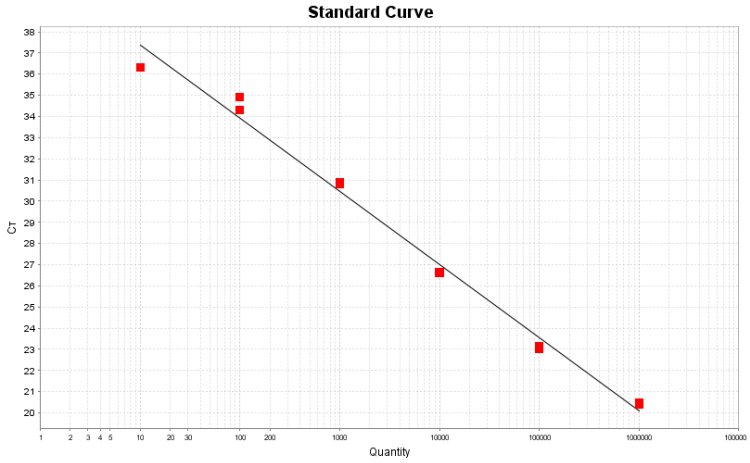

Figure 1. Detection of BCR/ABL transcripts using the GoTaq® 2-Step RT-qPCR System (Cat.# A6010). BCR/ABL transcripts were detected down to the presence of 100 K562 cells (both replicates) in 2.5ml of whole blood using the ENF501 and ENR561 primer sets (Gabert, et al., Leukemia 2003;17: 2318-2357). Slope = -3.453. R^2 = 0.989. Efficiency = 94.8%.

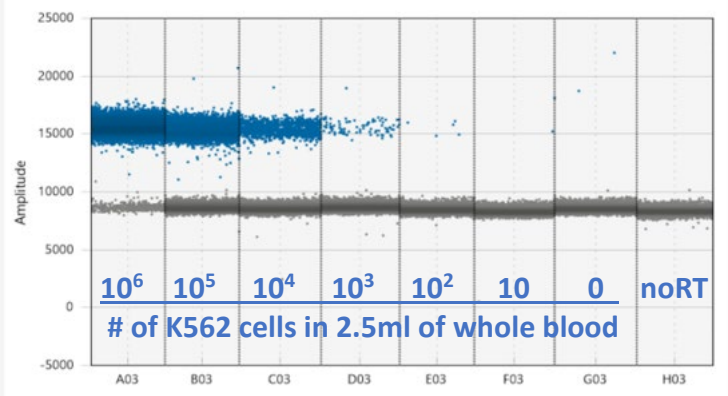

Figure 2. Detection of BCR/ABL transcripts in ddPCR. BCR/ABL transcripts were detected down to the presence of 100 K562 cells in 2.5ml of whole blood using the ddPCR EvaGreen Supermix (BioRad) with the BCR-ABL1 b3a2 primer sets (Stanoszek, et al., The Journal of Molecular Diagnostics, Vol. 15, No. 3, May 2013). BCR/ABL positive droplets are in blue. Negative droplets are in black.

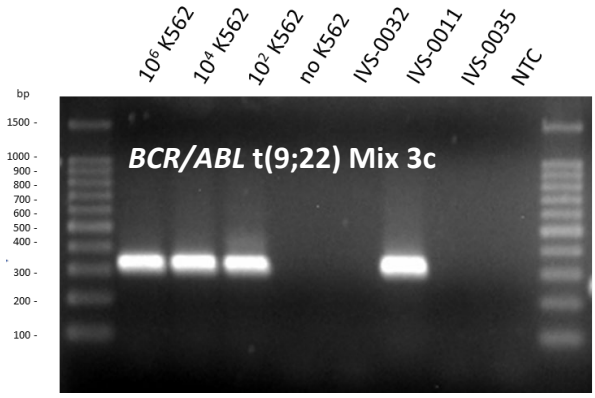

Figure 3. Detection of the p210 transcript using the BCR/ABL t(9;22) Translocation Assay (Invivoscribe). Varying numbers of K562 cells were spiked into 2.5ml of whole blood. Using Master Mix 3c, a 311bp product is expected for *BCR/ABL* positive samples for the p210 transcript. IVS-0032 = Clonal Control RNA (*BCR/ABL* p190 e1a2 Control RNA). IVS-0011 = Clonal Control RNA (*BCR/ABL* p210 b3a2 Control RNA). IVS-0035 = Clonal Control RNA (negative control for *BCR/ABL*). NTC = No template control. The size marker is the BenchTop 100bp DNA Ladder.