Promega

Product Application

Automated Purification of RNA for BCR-ABL Detection with BCR/ABL t(9;22) Translocation Assay from Invivoscribe

Purify RNA of sufficient quality and quantity for amplification using the Invivoscribe BCR/ABL t(9;22) Translocation Assay.

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

Analyses: BCR/ABL t(9;22) Translocation Assay

Sample Type(s): RNA from 2.5ml of human whole blood spiked with BCR-ABL positive K562 cells

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**

Materials:

- Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)
- Maxwell® RSC Instrument (Cat.# AS4500)
- BCR/ABL t(9;22) Translocation Assay (Invivoscribe, Cat.# 13100010)

Protocol: **RNA Purification**

1. Transfer 2.5ml of well mixed, fresh (not frozen) whole blood from the EDTA collection tube into a sterile 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 discard the supernatant with a pipette.
   Optional: As a positive control, add 100ul of 10^6, 10^4, 10^2, or no K562 cells to white blood cell pellets. Centrifuge tube again at 3,000 x g for 10 minutes.
6. Proceed with the standard protocol according to the Maxwell® RSC simplyRNA Blood Kit Technical Manual (TM417).

Protocol: cDNA Synthesis using the 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. Incubate reverse transcription reaction according to the GoTaq® 2-Step RT-qPCR System Technical Manual (TM337).

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

1. Generate cDNAs for control RNAs as described above using the GoScript™ Reverse Transcriptase.
2. Add 5µl of the resulting cDNAs (diluted 1:4 in Nuclease-Free Water) from test and control samples into each appropriate 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:

Quality of RNA for the Invivoscribe assay is assessed by targeting a region of the ABL transcript. This transcript is an internal amplification standard. Presence of the 94 base-pair amplification product using master mix 1b confirms: 1) there was RNA of sufficient quality and quantity to convert to cDNA, and 2) cDNA synthesis progressed from this RNA template in a manner sufficient to provide adequate material for PCR amplification of this control region. For all test and control samples, the 94bp product is detected, confirming that the RNA is of sufficient quality and quantity for amplification using this assay. The NTC sample was negative as expected.

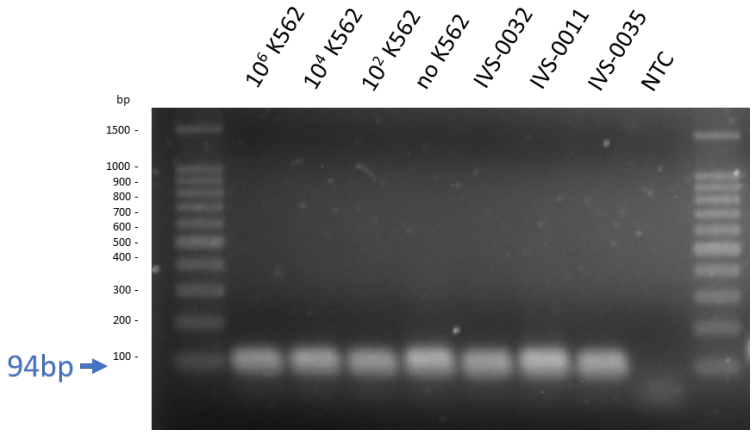

Figure 1. Amplification of the ABL gene as an internal control. Varying numbers of K562 cells were spiked into 2.5ml of whole blood. IVS-0032 = Clonal Control RNA (*BCR/ABL* p190 e1a2 Control RNA). IVS-0011 = Clonal Control RNA (*BCR/ABL* p210 b3a2 Control RNA). IVS-0035 = Clonal Control RNA (Negative control for *BCR/ABL*). NTC = No template control. The size marker is the BenchTop 100bp DNA Ladder.

Using mixture 3c, a 311bp product is expected for the p210 transcript with *BCR/ABL* positive samples. For all test samples containing *BCR/ABL* positive K562 cells and the control IVS-0011 sample, the 311bp product is detected, confirming that the p210 transcript is present and the RNA is of sufficient quality and quantity for amplification using this assay. The control IVS-0032 and IVS-0035 samples as well as the NTC sample were negative as expected.

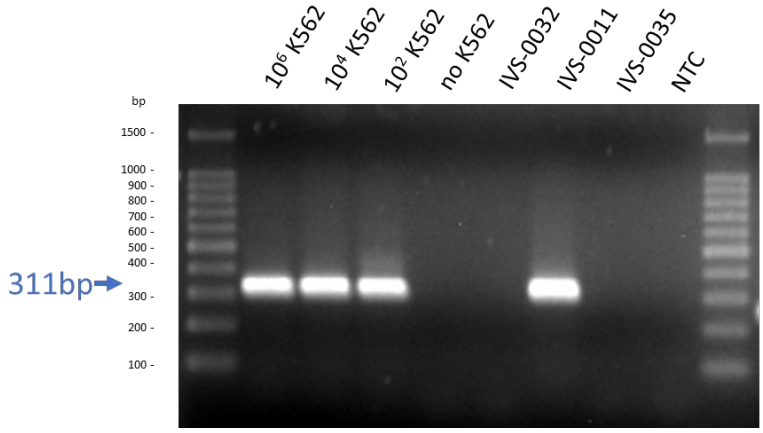

Figure 2. Amplification of the p210 transcript. Varying numbers of K562 cells were spiked into 2.5ml of whole blood. IVS-0032 = Clonal Control RNA (*BCR/ABL* p190 e1a2 Control RNA). IVS-0011 = Clonal Control RNA (*BCR/ABL* p210 b3a2 Control RNA). IVS-0035 = Clonal Control RNA (Negative control for *BCR/ABL*). NTC = No template control. The size marker is the BenchTop 100bp DNA Ladder.