

Direct amplification of purified mRNA from magnetic particles using QuickPick mRNA micro and nano kits

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ABSTRACT

Isolation of messenger RNA relies on the binding of poly-A(+) tails of the mRNA to oligo (dT) nucleotides on a carrier surface. Often, coated magnetic particles are used as such a carrier. This is also the design used in the QuickPick mRNA kits, which together with the PickPen® magnetic tool form a rapid and simple basis for mRNA purification. In addition, it has been shown that the isolated mRNA product still attached to the magnetic particles can be directly used in RT-PCR amplification without prior elution from the magnetic particles. This further increases the speed of the protocol and simplifies the process, while at the same time maximizing recovery, which can be of considerable importance when working with extremely small samples such as LCM (laser capture microdissection) samples.

INTRODUCTION

The QuickPick mRNA kits are ideal when working with small and limited amounts of starting material. For example LCM, where mRNA is isolated from a small number of cells, is one such application. When isolating mRNA from only a single cell it becomes particularly important to recover all of the mRNA present in the cell with only minimal losses.

PRINCIPLE OF THE QuickPick mRNA KIT

The QuickPick mRNA method is based on the binding of poly-A(+) mRNA to immobilized oligo (dT)₃₀ on paramagnetic particles. The sample is

first lysed in Lysis/Binding Buffer. The magnetic particles are washed once in the Lysis/Binding buffer and added to the lysed sample, followed by a 4-minute incubation at room temperature during which the poly-A(+) anneals to the oligo (dT) on the particles. The incubation is followed by three quick washes, first twice in Wash Buffer A and then once in Wash Buffer B to remove impurities. Finally the particles are transferred into the Elution Buffer. At this point, it is possible to elute mRNA from the magnetic particles into solution by heating at +70 °C for 5 minutes and removing the magnetic particles. It is also possible to use the mRNA still attached to magnetic particles directly as a template in the RT-PCR reaction, which simplifies and speeds up the process. The magnetic particles have been shown not to interfere with the RT-PCR.

MATERIALS & METHODS

The kit protocols (QuickPick mRNA micro or QuickPick mRNA nano) were followed as described in the kit inserts. The kits differ in the amount of recommended starting material, as well as in the reagent volumes used, but in all other respects the protocols are identical.

To prepare samples containing only few cells the instructions in the kit insert were followed. U937 cells grown in culture medium were harvested, counted, washed and diluted in Lysis/Binding Buffer to a concentration of 10 000 and 1000 cells per 200 µl for micro purifications and to a concentration of 50, 10 and 1 cell per 100 µl for nano purifications. Sample purifications were done according to the protocols in the kit inserts with PickPen® 1-M.

For mRNA nano purification analysis all of the Elution Buffer with magnetic particles (5 µl) was used directly in solid-phase amplification. Omitting the elution step shortened the protocol from approx 10 minutes to 5 minutes per purification. With the QuickPick mRNA nano kit only solid-phase amplification is recommended due to the small volumes.

To compare elution vs solid-phase results with mRNA micro kit the Elution Buffer with the magnetic particles was divided in two aliquots of 5 μ l. The first part was heated at +70 °C for 5 minutes after which the magnetic particles were removed and the eluate was used as a template in amplification. The other part was used in RT-PCR reaction directly.

The amplifications were carried out using Titan One Tube RT-PCR kit (Roche) and GeneAmp® PCR System 2700 (Applied Biosystems). Primer for U937 cell samples was 62-414 β -actin (350 bp). The amplification products were visualized on 1.5% agarose gels.

RESULTS

The pictures below show results using solid-phase or eluted mRNA samples as RT-PCR templates. mRNA was successfully amplified directly from magnetic particles, and is the protocol of choice when working with extremely small samples as no material is lost in the elution step.

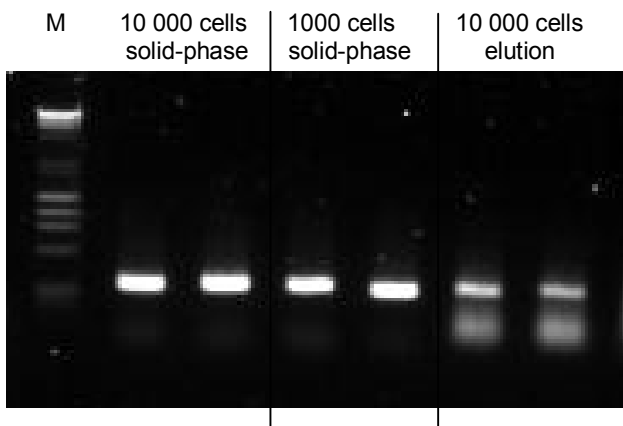


Figure 1: QuickPick mRNA micro kit, RT-PCR results from 10 000 and 1000 cultured cells on 1.5% agarose gel. With 10 000 cells RT-PCR reaction was carried out both with eluted and solid-phase mRNA. (Additional bands below the product are excess primer.)

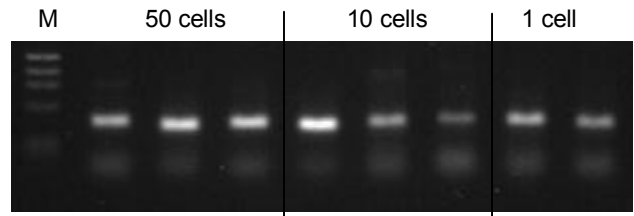


Figure 2: QuickPick mRNA nano kit, solid-phase RT-PCR results from 50, 10 and 1 cultured cell on 1.5% agarose gel.

CONCLUSIONS

The QuickPick mRNA micro and nano kit protocols may be shortened from approximately 10 minutes to 5 minutes per preparation by omitting the final elution step. The purified mRNA remains bound to the oligo (dT) coated magnetic particles. This protocol is useful in particular when the downstream application is RT-PCR. This saves time and ensures that as much as possible of the purified mRNA is available for the RT-PCR amplification.

PickPen 8-M can be used with equally good results, and further increases the throughput, as 8 samples can be processed in parallel on one microplate.