Application Note

QIAscout® CTC single cell isolation after AdnaTest CancerSelect pre-enrichment

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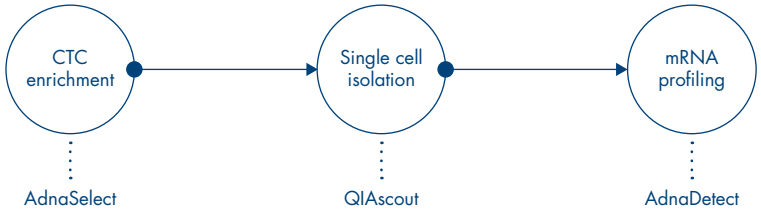
This application note demonstrates that single circulating tumor cells (CTCs) that have been pre-enriched with AdnaTest ProstateCancerSelect technology are suitable for isolation using the QIAscout system. Furthermore, the single CTCs isolated with the QIAscout system are compatible with downstream mRNA profiling using AdnaTest ProstateCancerDetect.



Introduction

Single cell technologies can provide deeper insights into all facets of tumor states and behaviors, including intra-tumor heterogeneity, clonal evolution in primary tumors, invasion in early stage cancers, epithelial-mesenchymal transition and metastatic dissemination. This technology allows researchers to profile the genome of CTCs, to investigate mutation rates and phenotypes and to understand resistance evolution to therapy.

The ability to isolate and evaluate CTCs and their patient-specific expression profile is promising both for diagnosis and clinical management as for monitoring individual treatment. A blood sample, a callled "liquid biopsy" sample, can be used by clinicians for prognostic and predictive purposes.

While the total amount of CTCs in the blood varies from 1 to 10 CTCs per milliliter, the amount of leukocytes is very high (approximately 10^7 per milliliter). It is known that CTCs can be subdivided into different types, such as cytokeratin-positive CTCs, stem cell CTCs, apoptotic CTCs and small CTCs. Analysis of single CTCs would allow the detailed characterization of ▷

such individual CTC types without interference from other CTCs or contaminating leukocytes. For research and clinical applications this also allows for higher sensitivity in downstream applications, such as next generation sequencing (NGS) or any other technique, and a reduced rate of false negative or false positive results.

AdnaTest technology enables reproducible and standardized analysis of CTCs in blood. Neither a special instrument nor large investment is required. Fast analysis shortens time to results while high sensitivity and resolution bring confidence to turn results into insights. The AdnaTest is easy to establish in any lab. Surface antigen expression on tumor cells is highly variable and can lead to false negatives due to epithelial-mesenchymal transition (EMT) or the development of a tumor stem cell phenotype. AdnaTest Select enables the immunomagnetic enrichment of tumor cells using an optimized combination of epithelial and tumor associated antibodies. The combination of different antibodies for tumor cell enrichment profoundly increases the analytical sensitivity and helps to avoid false negative results.

Variability of tumor cell expression and variations during therapy and at different time points of sample collection lead to unpredictable expression patterns, which may in turn lead to false-negative results. The AdnaTests resolve this problem by using a combination of tumor markers at the mRNA level.

Altogether, AdnaTest technology combines a highly specific immunomagnetic cell selection of CTCs, using an optimized antibody combination, with a highly sensitive RT-PCR technology, using a combination of mRNA tumor markers. This combination ensures the required clinical specificity and sensitivity for a valid diagnostic application.

The QIAscout instrument (Figure 1) is ideal for isolation of single cells of various cell types, such as adherent or suspension cells, primary cells or cell lines, and fluorescent cells. Additionally, the QIAscout 12,000-Microraft Array provides all cells with a suitable environment for growth and viability similar to any standard cell-culture dish. Microrafts carrying individual cells can be pierced, dislodged and transferred to reaction tubes using a magnetic wand, without risk of cross-contamination at any given stage. Subsequently the cells can be further processed using a variety of downstream applications, such as NGS, or even be further cultivated.

Figure 1. The QIAscout system.

Steps in the QIAscout workflow include:

- Seeding and cultivating cells in medium on the QIAscout array
- Placing release device containing release needle on the microscope objective
- Placing array on the microscope stage
- Identifying microrafts containing single cells
- Piercing microraft containing cell of interest
- Transferring microraft to secondary vessel using a magnetic wand
- Processing the collected cell for downstream application or further cultivation

Material and Methods

As a model system to demonstrate the combination of immunomagnetic enrichment of CTCs followed by QIAscout single cell isolation and multiplex RT-PCR for expression analysis of tumor marker mRNA, 20 LnCAP95 cells were spiked into 5 ml of healthy donor blood.

Tumor cell enrichment was performed using the AdnaTest ProstateCancerSelect via immunomagnetic beads labeled with anti-EpCam and anti-Her2 antibodies. Tumor cells were separated with a magnetic particle separator (AdnaMag-L), and residual red blood cells and most leukocytes were removed during several PBS washing steps.

In deviation to the AdnaTest ProstateCancerSelect workflow the resulting magnetic bead cell mixture was then applied to the QIAscout array. The QIAscout array was pretreated with CellTak (Corning, according to manufacturer's protocol including a 20-minute incubation step) to allow attachment of the tumor cell/bead aggregate. After applying 2 ml of cell culture medium (RPMI 1640 without phenol red and glutamine, 10% FCS charcoal serum, 1% penicillin/streptomycin) to the QIAscout array, the bead/cell mixture from the AdnaTest Select procedure was added. When the CTCs were attached to the array surface (after approximately 4 hours), the entire array was screened for the presence of CTCs. For better visual inspection, residual empty magnetic beads were removed from the QIAscout array by gently moving the magnetic wand over the array. Altogether, this procedure allowed for reliable identification and isolation of single tumor cell/bead aggregates under microscopic control for subsequent mRNA profiling.

Further processing was performed as described in the instructions for the AdnaTest ProstateCancerDetect (QIAGEN, Hilden, Germany). In brief, the isolated cells were lysed in 200 µl AdnaTest Lysis/Binding Buffer, and mRNA was purified using Oligo(dT) magnetic beads. The resulting mRNA was reverse transcribed to cDNA (Sensiscript® RT Kit, QIAGEN, Hilden, Germany) and subsequently analyzed in a multiplex endpoint PCR for tumor associated transcripts (PSMA, PSA, EGFR) and cellular control (actin).

Results and Discussion

All cells that were spiked into the healthy donor blood were captured via the AdnaTest ProstateCancerSelect workflow and detected on the QIAscout array. Figure 2 shows an example of a single tumor cell/bead aggregate located on a raft of the QIAscout array. CTCs could be easily distinguished from leukocytes (Figure 3) by their morphology and size. No raft contained more than one cell, leading to no doublets.

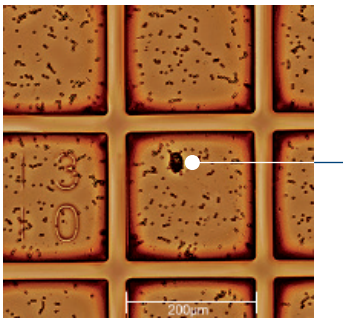


Figure 2. Tumor cell/bead aggregate on QIAscout array.

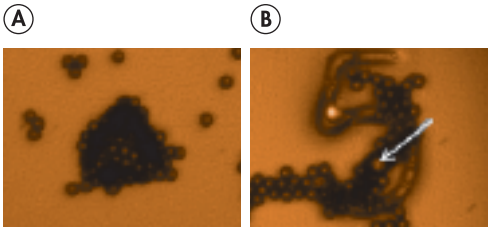


Figure 3. Tumor cell/bead aggregate (A) and potential leuokocyte (B, indicated by arrow) in same scale.

Seven rafts, each containing a single cell of interest, were selected for isolation using the QIAscout system. Subsequent mRNA profiling was successful for all cells analyzed, as shown by the expression of actin in all samples (Figure 4). This indicates that the transfer rate of cells from the QIAscout array to the reaction tube was 100% and that residual magnetic beads do not interfere with the AdnaTest ProstateCancerDetect workflow.

mRNA profiling of tumor associated transcripts demonstrated that 6 out of 7 isolated tumor cells were positive for the presence of the PSMA tumor marker (indicated in Figure 4). LnCAP95 cells are known not to transcribe the EGFR gene, as indicated by the absence of EGFR transcripts (Figure 4). The heterogeneous character of tumor marker expression in individual CTCs is reflected by the absence of PSA transcription for all tested samples and the heterogeneous expression of the PSMA gene (Figure 4).

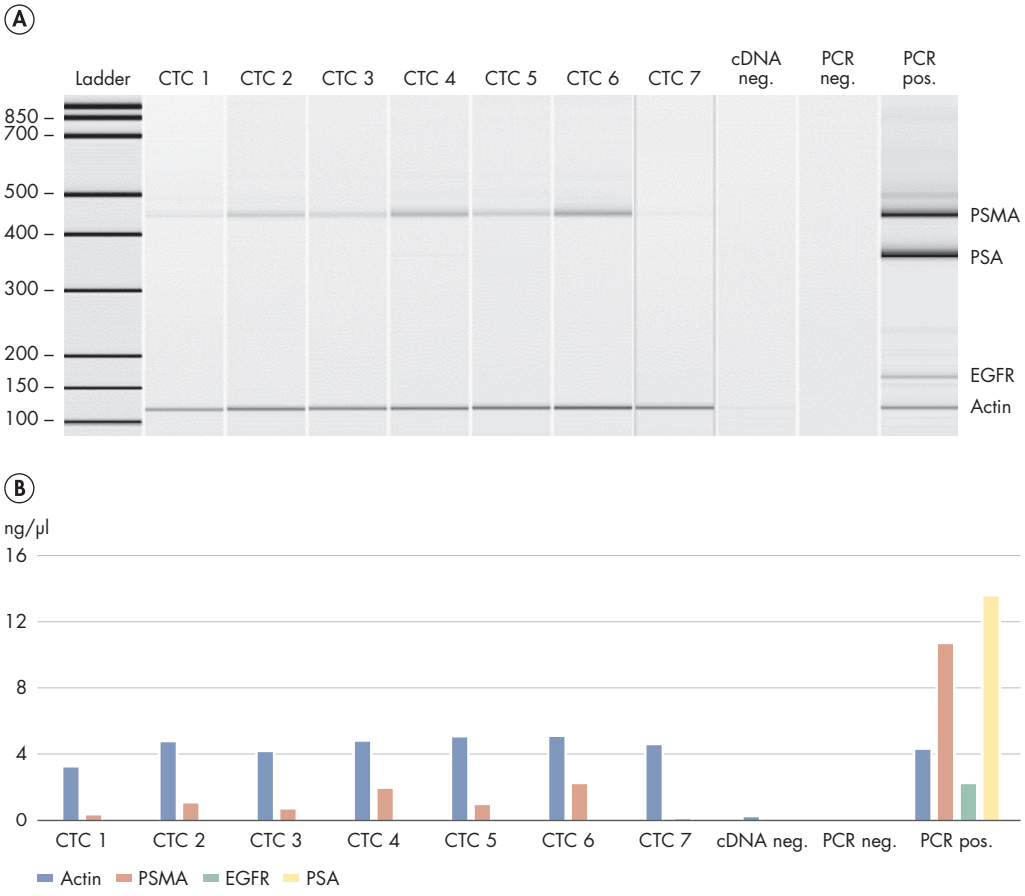


Figure 4. Results of AdnaTest ProstateCancerDetect. Expression pattern of three tumor markers (PSMA, PSA, EGFR) and one cell marker (actin) is shown. **A** Capillary gel-electrophoresis picture. **B** Bar chart of results with transcript levels as expressed in ng/µl PCR product.

Conclusion

- The AdnaTest ProstateCancerSelect workflow efficiently enriches tumor cells that have been spiked into blood from healthy donors.
- Tumor cell/bead aggregates are compatible with the QIAscout array and can easily be identified and distinguished from other cell types by morphology and size.
- QIAscout technology allows efficient and reliable isolation of single tumor cells.
- AdnaTest ProstateCancerDetect is sensitive enough for detection of tumor marker transcripts derived from a single cell.
- Altogether, the combination of the AdnaTest ProstateCancer Test and the QIAscout system is a powerful tool for analysis of single CTCs, which helps to elucidate the heterogeneous character of single cells and the analysis of rare cells.

Ordering Information

Product	Contents	Cat. no.
QIAscout	Includes instrument platform and starter pack of 5 arrays	9002733
QIAscout 12,000-Microraft Arrays	5 arrays	928031
AdnaTest ProstateCancerSelect	For isolation of CTCs and subsequent extraction of mRNA from human whole blood for 12 preparations	Inquire, varies between countries
AdnaTest ProstateCancerDetect	RT-PCR kit for detection of prostate cancer-associated gene expression in enriched tumor cells	Inquire, varies between countries

The QIAscout is intended for molecular biology applications. The applications described here are not intended for diagnostic use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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