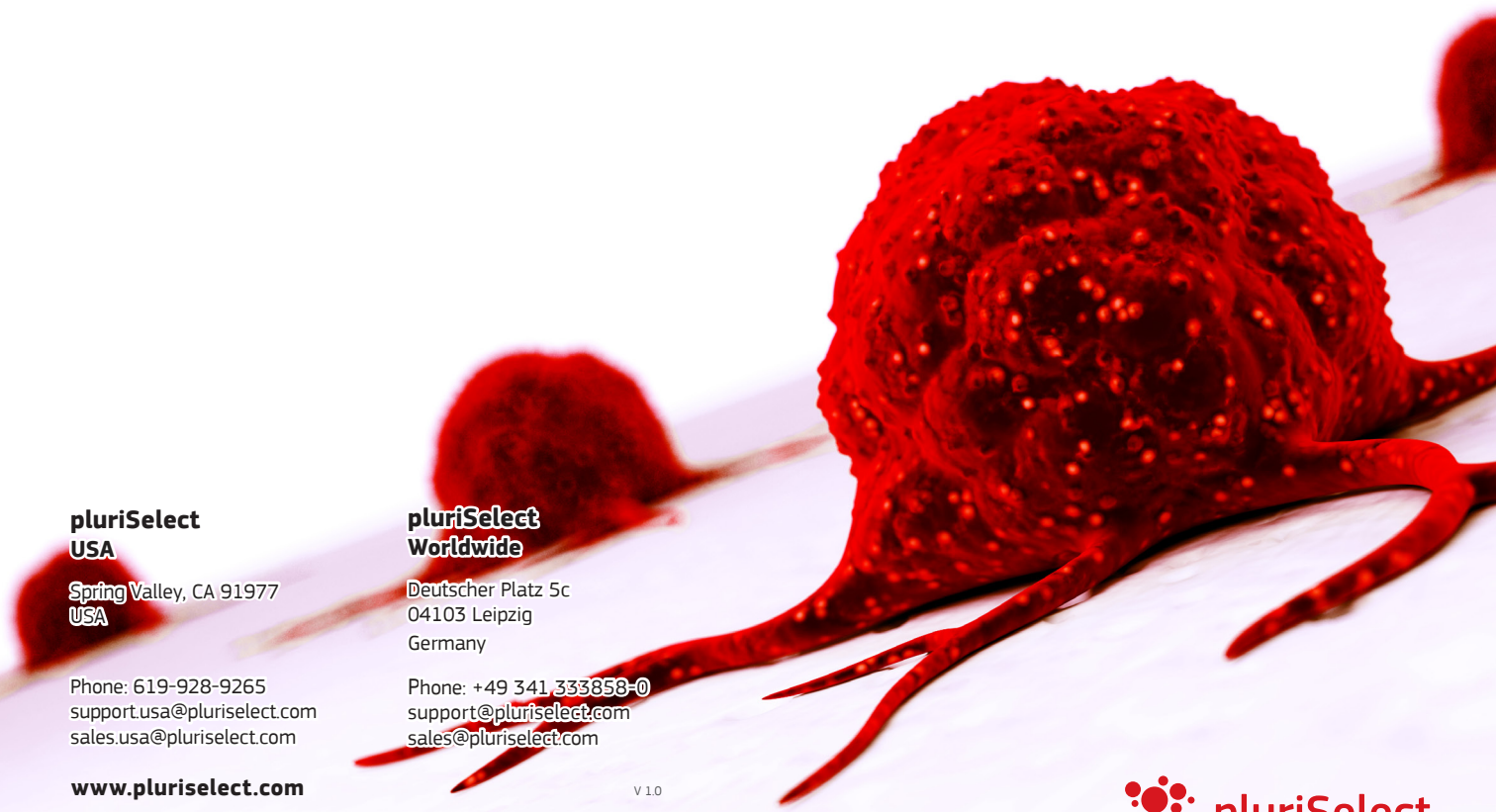
FAST, EASY, GENTLE!

pluriBead®

Application Example

Isolation of rare cell types: Circulating tumor cells



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V 1.0

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Isolation of rare cell types: Circulating tumor cells

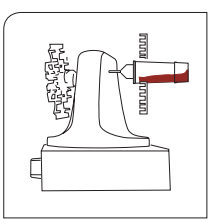
Introduction

Here we are presenting a new approach to colon carcinoma circulating tumor cell (CTC) screening using pluriBead® carrying a tumor-associated EpCAM antibody.

This method is based on a non-magnetic cell separation technology. It does not require any sample pre-treatment. EpCAM-pluriBead® can be added directly to a whole blood sample. The method is also suitable for single cell isolation from different biological fluids. Moreover, its sensitivity can be additionally increased by raising the sample volume. The bound EpCAM-positive colon carcinoma cells can be easily involved in further molecular-genetic experiments that aim to detect of their mutation status. In case of colon carcinoma, K-ras mutation status is a predictive tool of response anticancer therapy. Thus, the new method can be considered as a fast and effective instrument for early cancer diagnostics.

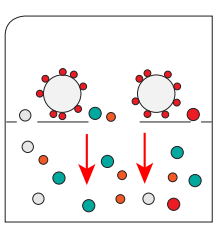
Materials and methods

A: Labeling
Add EpCAM-pluriBead® to your sample.

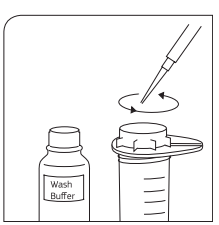


B: Incubation
30 minutes of gentle incubation (recommended with pluriPlix®).

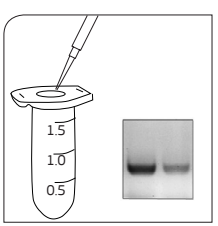
C1: Separation
Captured target cells are separated via appropriate sieves.



C2: Separation
Target cells bound on pluriBead® stay on top of the sieve. The rest runs through.



D: Washing & lysis
Use wash buffer to clean sieve. Lyse cells with Trizol®.



E: Processing
Approaches for the study of cancer cells:
- RNA/DNA isolation
- Cell culture experiments

Results

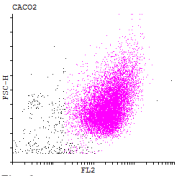


Fig. 1a

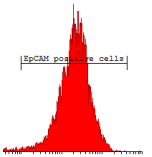


Fig. 1b

Fig. 1a and 1b: Flow cytometric analysis of Caco-2 cells, staining with anti-EpCAM-PE.

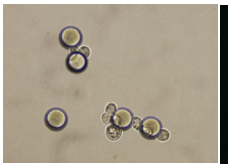


Fig. 2a

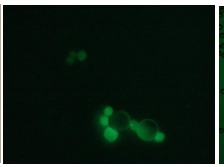


Fig. 2b

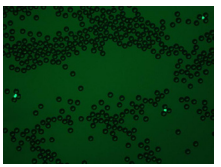


Fig. 2c

2a) Colon carcinoma Caco-2 cells captured by EpCAM-pluriBead®.
2b) Captured colon carcinoma Caco-2 cells, staining with calcein.
2c) Captured colon carcinoma Caco-2 cells spiked in 30 ml whole blood with 0.5 million EpCAM-pluriBead®.

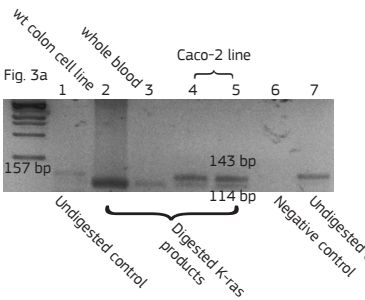


Fig. 3a

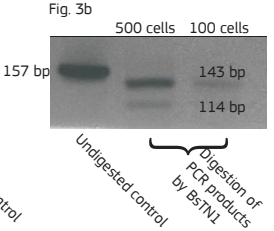


Fig. 3b

Fig. 3: Screening of colon carcinoma lines for the presence of mutation in 12h codone of K-ras oncogene by PCR using primers that introduce BsTN1 restriction enzyme sites into PCR products.

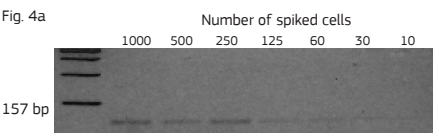


Fig. 4a

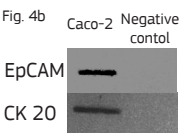


Fig. 4b

Fig. 4a: Sensitivity of PCR method for the detection of K-ras protoncogene in known number of spiking Caco-2 cells. Fig. 4b: Products of RT-PCR for EpCAM and CK 20 mRNA isolated from the Caco-2 cells, captured by EpCAM-pluriBead® (as a negative control, mRNA from whole blood was used).

Conclusions and perspectives

Here we developed a new approach for the detection of circulating colon tumor cells in blood using EpCAM-pluriBead®. Captured cells are suitable for further molecular-genetic screening of specific markers, connecting with tumor formation. The developed “in situ immunobeads pcr” method does not require preliminary RNA/DNA isolation and can effectively save the time of analysis. The further aim of this work is to increase the sensitivity of method raising the sample volume, varying the cell tumor lines and/or antibody specificity.