A Method for Detecting Protein Expression in Single Cells Using the C1™ Single-Cell Auto Prep System

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Introduction
Recent improvements in microfluidics and biochemistry have enabled single-cell molecular analysis, providing new insight into the heterogeneity of cell populations. The C1™ Single-Cell Auto Prep System is an automated platform that streamlines the isolation and processing of 96 individual, live cells for RNA and DNA analysis. Single-cell protein profiling is a direct complement to genomic analysis as it provides additional insights into key molecular mechanisms and system biology. To enable this, we adopted a highly multiplexed protein detection method (Proseek Multiplex Oncology I™), based on the Proximity Extension Assay (PEA) for use on the C1™ Single-Cell Auto Prep System.

Overview of the C1™ Single-Cell Auto Prep System for Protein Detection
We have used the C1™ Single-Cell Auto Prep System in combination with the Proximity Extension Assay technology (PEA, Figure 1A) to develop a workflow for the automated analysis of the protein expression of single cells (Figure 1B). The method developed is based on the use of a PEA probe panel targeting 92 different proteins and of those 66 correspond to intracellular proteins that can be detected in single cells (Figure 1E).

**Results**

**Protein Detection in Single Cells is Consistent Across Chip and Plate Experiments**
Figure 5 Results from PEA on plate-sorted cells were compared to results obtained from two independent C1™ PEA experiments on single HL60 cells. In general, results obtained from plate PEA on sorted cells confirmed results obtained by C1™ PEA, with the exception of the Tissue Factor. However, plate PEA signal for this specific target does not increase as expected when 10 and 50 cells are tested, suggesting that the high background signal of plate PEA could be affecting expression level results for this method.

**Flow Cytometry and Immunofluorescence Results are Consistent with C1™ PEA Results**
Figure 6 A, C1™ PEA results for two specific targets were validated on HL60 and K562 cells using orthogonal methods: EpCAM (low and high expression, respectively) and EMMPRIN (high expression in both cell types) antibodies conjugated with fluorescent dyes were used to evaluate expression levels of populations of cells with flow cytometry (Flow) and for on-chip immunofluorescence (IF) on single cells prior to C1™ PEA. Flow and IF results were highly concordant with PEA results and some of the expression rate differences observed can be explained by different antibodies used across the methods and different populations of cells tested (Flow vs. PEA and IF). B, The diagram shows a heat map of characteristic protein expression results for C1™ PEA and IF for EpCAM (red indicates high expression). As expected, K562 cells have high EpCAM expression confirmed by PEA and IF and HL60 cells have high MPO expression levels confirmed by PEA. Two cells out of 38 analyzed with IF and PEA had results different than expected, presenting both EpCAM expression (IF and PEA) and MPO (PEA). For one of those cells it has been confirmed that two instead of one cell had been captured in the C1™ IFC chamber (panel C).

**Conclusion**
• We have developed methodology for automated protein detection from single cells on the C1™ Single-Cell Auto Prep System, with the ability to simultaneously perform triplicates on different cell lines.
• The method is sensitive enough to detect expression levels from single cells and is a promising technique to use in combination with DNA and RNA profiling from single cells for further system biology studies. It is also consistent with other studies that target gene expression (References).
• The PEA probe panel from the Proseek Multiplex Oncology I™ kit, which targets 92 potential cancer-related targets, has been successfully used in profiling single cells derived from both cancer and normal tissue, grouping 98% of all cells analyzed (n=401).

**References**

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