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Unleashing Single-Cell Proteomics: scPICO Revolutionizes Absolute Quantification with F.SIGHT™ & I.DOT Technology

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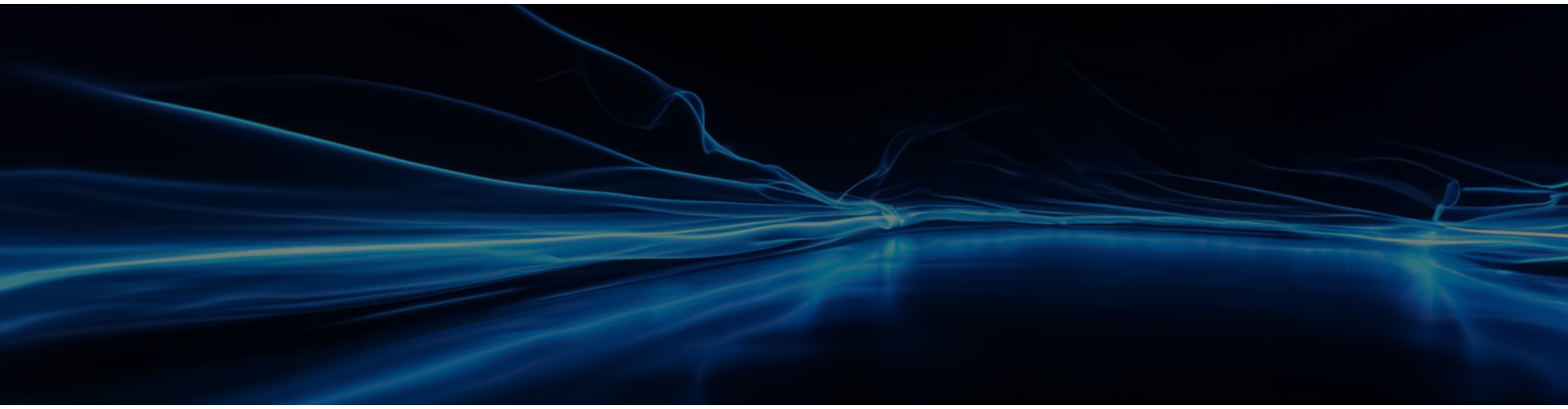

The Need for Accurate, Efficient Single-Cell Proteomics Technologies in Research and Development

Protein quantification is a crucial technique utilized by scientists across various research fields, from cancer research to drug discovery. Traditionally, immunoassays such as Western blotting and ELISA have been employed due to their simplicity and cost-effectiveness. However, in their conventional formats, these assays have limitations in sensitivity, can be affected by sample complexity, and are labor-intensive, making them prone to human error and unsuitable for high-throughput applications[1–5].

Other protein quantification methods, such as mass spectrometry and flow cytometry, also face challenges. The sample preparation for these assays is often complex and time-consuming, and the high costs associated with mass spectrometry limit its accessibility to many laboratories[6–8].

With the growing understanding of the heterogeneous cellular and molecular landscape within tissues in healthy and diseased states, there is an increasing need to explore protein distribution at the single-cell level[9–11]. Although most of the techniques can be adapted for single-cell analysis, they still suffer from the same limitations and can lack the throughput required for comprehensive single-cell studies. Therefore, there is a significant need for a highly sensitive, efficient, and high-throughput methodology for accurate protein quantification at the single-cell level.

The Single-Cell Protein Interaction Coupling Assay

To overcome these challenges and help scientists achieve accurate, sensitive, and specific protein quantification in single cells, Actome has developed the single-cell protein interaction coupling (scPICO) assay. The scPICO assay represents a significant advancement in single-cell proteomics, supporting reference-free absolute quantification (AQ) of proteoforms. The technology enables the precise detection and quantification of proteins, post-translational modifications, and protein-protein interactions at the single-cell level. The assay offers excellent sensitivity, with a high signal-to-noise ratio and minimal background interference.

Key Technologies

F.SIGHT™ Single-Cell Dispenser

In the scPICO assay, the F.SIGHT™ Single-Cell Dispenser accurately dispenses individual cells into a 384-well plate, ensuring that each well contains a single cell for analysis. The F.SIGHT™ Single-Cell Dispenser integrates rapid and precise cell isolation with gentle handling and contamination prevention to maintain cell integrity and support the high sensitivity and specificity of the scPICO assay.

Key Features, Advantages, and Benefits

1. Fast and Precise Dispensing

- **Feature:** Isolates single cells in 384-well plates, filling an entire plate in 7 minutes, containing the single cell in a droplet volume smaller than 200 picoliters.
- **Advantage:** Significantly reduces the time required for single-cell isolation and the chances of contamination by dispensing single-cell in picoliter volume droplets.
- **Benefit:** Accelerates experimental workflows, enhancing productivity.

2. Precise Isolation of Single Cells of Interest

- **Feature:** Equipped with an adjustable blue laser to detect subtle signals, the dual imaging system overlays brightfield and fluorescent images for precise cell identification, capturing images for each run.
- **Advantage:** Ensures isolation of only single cells of interest.
- **Benefit:** Improves the reliability and accuracy of single-cell proteomics assays, giving researchers confidence in their results.

3. Gentle Cell Handling

- **Feature:** Utilizes no-pressure dispensing.
- **Advantage:** Minimizes stress on delicate cells.
- **Benefit:** Preserves cell viability and function, crucial for downstream proteomics analysis.

4. Contamination Prevention

- **Feature:** Compact device that uses single-use cartridges and can be operated remotely from outside the sterile hood.
- **Advantage:** Ensures sterile conditions during isolation, eliminating the risk of contamination.
- **Benefit:** Maintains sample integrity and purity.

The scPICO Workflow

The scPICO workflow is a streamlined six-step process designed to overcome the challenges of traditional protein quantification assays (***Fig. 1***).

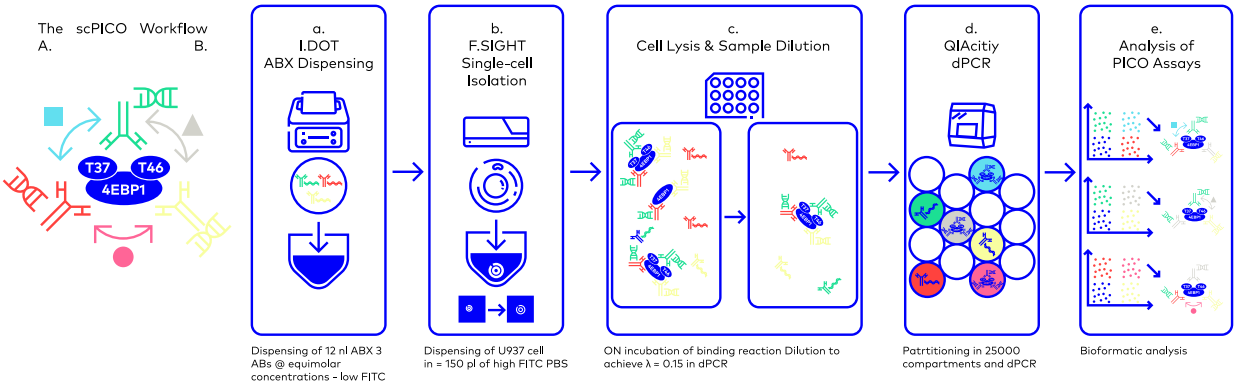

Figure 1. *The scPICO workflow.*

A. Triangular PICO assay, i.e., three assays are set up using pairwise readout of three concurrently binding 4EBP1 (EIF4EBP1 4F3-H2, Invitrogen; 4EBP1 60246-1-Ig, Proteintech) and p-4EBP1 (Phospho-4EBP1 4EB1T37T46-A5, Invitrogen) antibodies at 5.56e-11 M. The assays are indicated with arrows and corresponding colors, while the colors of antibodies match the dPCR fluorescent color channels. The 4EBP1 protein is read using red and yellow antibodies, while phosphorylation of 4EBP1 is measured by two pairs (self-confirmatory assay) red-green and green-yellow, respectively. **B.** ***a.*** Dispensing 12 nL volume of the antibody mix (ABX) in lysis buffer (ACTOME LBTW) under hydrophobic oil (Qiagen Vapor-Lock) in a 384-well plate (eppendorf Microplate 384/V-PP), using the DISPENDIX I.DOT Non-Contact Dispenser, ***b.*** and isolating optically interrogated single U937 cells in 150 pL volume and into the 384-well using the Cytena F.SIGHT™ single-cell dispenser. The previous two steps were fluorescently traced by FITC, to judge successful liquid handling steps. ***c.*** After overnight incubation **(*d*)** 2% of the material of the single cell was dPCR amplified on the QIAcuity system, and **(*e*)** PICO data analysis was carried out to gain the absolute number of detected copies of proteoforms in the dPCR volume.

1. **Antibody Preparation.** Antibodies are labeled with unique DNA oligonucleotides known as PICO labels. These labeled antibodies remain stable for six months and are suitable for multiple assays.
2. **Antibody Dispensing.** The labeled antibodies are accurately dispensed into 384-well microplates using the I.DOT Non-Contact Dispenser.
3. **Single-Cell Isolation.** Single cells are isolated from biological samples and dispensed into microplates using the F.SIGHT™ Single-Cell Dispenser.
4. **Incubation.** The single cells are incubated with the labeled antibodies overnight, leading to the formation of 'couplexes' – target proteigins bound by the two labeled antibodies.
5. **dPCR Amplification.** The sample is then diluted, mixed with matching PICO probes, and transferred to a dPCR plate for amplification.
6. **Data Analysis.** Data analysis is conducted to determine the precise absolute number of detected copies of proteoforms.

This study applied the scPICO assay to analyze 4EBP1 protein levels and phosphorylation (p-4EBP1) in single U937 cells. U937 cells were treated with the dual PI3K/mTOR inhibitor dactolisib and compared to bulk PICO. The performance of scPICO was assessed in terms of its single-cell sensitivity, and its ability to provide AQ measurements and reveal cellular heterogeneity was evaluated. The reliability of scPICO was further analyzed through validation studies.

Results

Single-Cell Sensitivity

U937 cells were treated with dactolisib (5.6 µM) for 4 hours or left untreated (DMSO) and subjected to the scPICO workflow to evaluate single-cell sensitivity. Dactolisib treatment led to a significant reduction in p-4EBP1 signals but did not affect the 4EBP1 protein signal. The analysis revealed marked heterogeneity in the treatment response among the population, supporting the assay's capability to detect variations at the single-cell level (***Fig. 2A***).

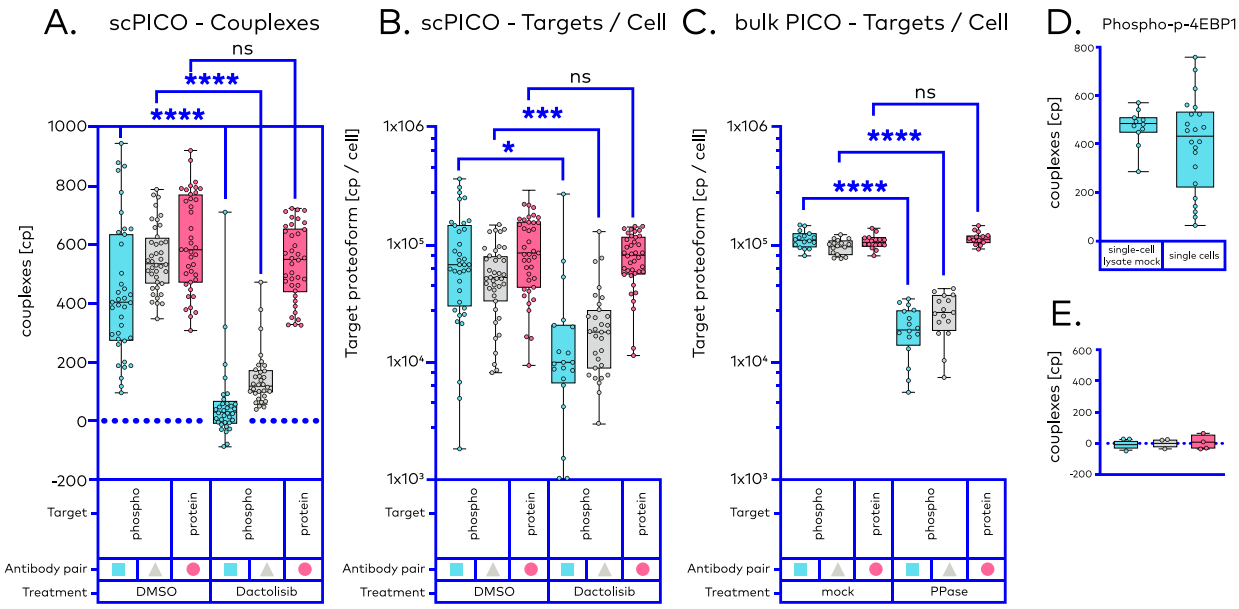

Figure 2. *Comparing Bulk and Single-cell PICO.*

A. scPICO - copies of detected couplexes. scPICO assesses a single cell in a 12 nL volume. U937 cells were treated with dactolisib (5.6 µM) for 4 h or left untreated (DMSO) and subjected to the scPICO workflow. Dactolisib treatment led to a significant reduction in p-4EBP1 signals but not in the 4EBP1 protein signal. Marked heterogeneity in the treatment response was observed among the cell population. **B.** scPICO - corresponding AQ signal of target proteoforms. **C.** Bulk PICO assays - 20,000 cells in a 4 uL volume, measuring absolute quantitative amounts (AQ) of 4EBP1 and p-4EBP1 in U937 bulk lysate at 5e-10 M of antibody concentration, treated with λ-phosphatase (PPase) [6] or left untreated (mock). Untreated samples showed comparable 4EBP1 and p-4EBP1 AQ levels, while λ-phosphatase treatment significantly reduced the p-4EBP1 signal, as expected. **D.** Evaluation of the true heterogeneity of scPICO. p-4EBP1 AQ levels in U937 single cells were compared to equivalent amounts of bulk lysate, both analyzed using the scPICO workflow depicting the raw couplex signal (single-cell lysate mock). **E.** ip-ABC - in-plate negative antibody binding control, using 12 nL volume ABX processed analog to single cells in scPICO but no cell added demonstrating zero signal with no background.

Comparative Analysis

The AQ signals of target proteoforms were compared between the scPICO assay (***Fig. 2B***) and bulk PICO assay (***Fig. 2C***). The bulk assay demonstrated that untreated samples had comparable AQ levels of 4EBP1 and p-4EBP1, while treatment with λ-phosphatase (PPase) significantly reduced the p-4EBP1 signal, comparable to the results observed with dactolisib treatment in the scPICO assay. This comparison highlights the robustness of scPICO in detecting treatment effects at the single-cell level.

Validation

Validation of the scPICO results was performed using multiple approaches. Western blotting confirmed the effect of dactolisib on p-4EBP1, verifying the reduction observed in scPICO assays. Additionally, the true heterogeneity of scPICO was validated by comparing p-4EBP1 AQ levels in U937 single cells to equivalent amounts of the bulk lysate (***Fig. 2D***). Finally, an in-plate negative antibody binding control (ip-ABC) without added cells demonstrated zero signal with no background, confirming the scPICO assay's specificity and accuracy (***Fig. 2E***).

Discussion

The results of this study demonstrate the exceptional capabilities of the scPICO assay in providing highly sensitive and accurate AQ measurements of 4EBP1 and p-4EBP1 in single U937 cells. The scPICO assay successfully identified significant heterogeneity in the cellular responses to dactolisib treatment, highlighting its high sensitivity compared to bulk assays. This sensitivity is critical for uncovering cellular behaviors that are masked in bulk analyses, providing deeper insights into population dynamics and treatment responses.

The integration of the I.DOT Non-Contact Dispenser and the F.SIGHT™ Single-cell Dispenser likely played a pivotal role in the success of the scPICO assay. The I.DOT's precision and speed in dispensing minute volumes of reagents ensure efficient and accurate preparation of antibody solutions, which is critical for maintaining the assay's sensitivity and specificity. Its non-contact dispensing technology also minimized the risk of cross-contamination, ensuring the integrity of single-cell samples.

The F.SIGHT™ Single-cell Dispenser facilitates the precise isolation of single cells. Its gentle cell handling preserves cell viability, while the dual imaging system ensures accurate identification and isolation of target cells, crucial for achieving reliable and reproducible results in the scPICO workflow.

Furthermore, the scPICO assay's validation through Western blotting and comparison with bulk PICO results confirmed its reliability and analytical performance. The ability to detect true heterogeneity in p-4EBP1 levels among single cells, validated by control experiments, highlights the assay's potential for enhancing our understanding of how cellular heterogeneity impacts treatment outcomes.

Conclusion

In summary, scPICO is an innovative single-cell assay that delivers highly sensitive and accurate AQ measurements. This study highlighted its ability to effectively quantify 4EBP1 and p-4EBP1 levels with zero background noise and reveal significant heterogeneity in cellular responses, which is likely critical for personalized medicine. The integration of the I.DOT and F.SIGHT™ dispensers were essential for the assay's precision and reliability. This breakthrough technology holds significant promise for advancing targeted therapies, especially in cancer treatment, where cellular heterogeneity plays a crucial role.

Acknowledgments

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