TECHNICAL NOTE

Optimizing Cell Viability for Single-Cell Sample Preparation

Optimizing cell viability during enrichment, stimulation, staining, and loading of samples into IsoCode Chips.

In this Technical Note we outline:

- Maximizing cell viability in single-cell runs
- Identifying sources of low cell viability
- Sample viability required prior to loading on chip
- Data normalization based on sample viability

BRUKER

Introduction

The Bruker's walk-away proteomics platforms allow for single-cell biology connected with highly multiplexed functional proteomics for the first time. The IsoLight and IsoSpark's built-in incubator, advanced fluidics, and precision imaging, combined with the IsoSpeak software platform allows users to visualize, target, and utilize data from direct, functional immune landscaping, correlating with *in vivo* biology.

In contrast to the labor intensive workflow of traditional proteomics systems, the Bruker platforms require minimal amount of hands-on time for successful measurement of true function from single cells. In this Technical Note, we will cover several key guidelines and tips for the optimization of cell viability for consistent data on your IsoLight and IsoSpark Systems.

Sample preparation workflow

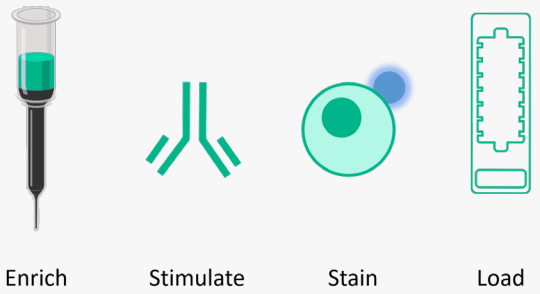

Figure 1 | Sample preparation workflow. 1) sample enrichment, 2) cell stimularion, and 3) cell staining prior to loading onto the IsoCode chip.

Maximizing Cell Viability in Single-Cell Runs

Before loading samples onto IsoCode chips and running on the IsoLight or IsoSpark, four simple steps are required (Figure 1):

1. **Sample Enrichment**
2. **Cell Stimulation**
3. **Cell Staining**
4. **Cell Loading**

Once the cells are loaded onto IsoCode chips and into Bruker's systems, all downstream proteomic workflows are performed by the system in an automated fashion. Ensuring sample viability prior to loading the cells into the IsoCode chip will play a critical role in the overall quality of functional single-cell analysis.

Within each of the four steps in the sample preparation process (Enrichment, Stimulation, Staining, and Loading), there are a few critical tips to ensure optimal viability of the sample (Figure 2).

Protocol cautions to maximize viability during sample preparation

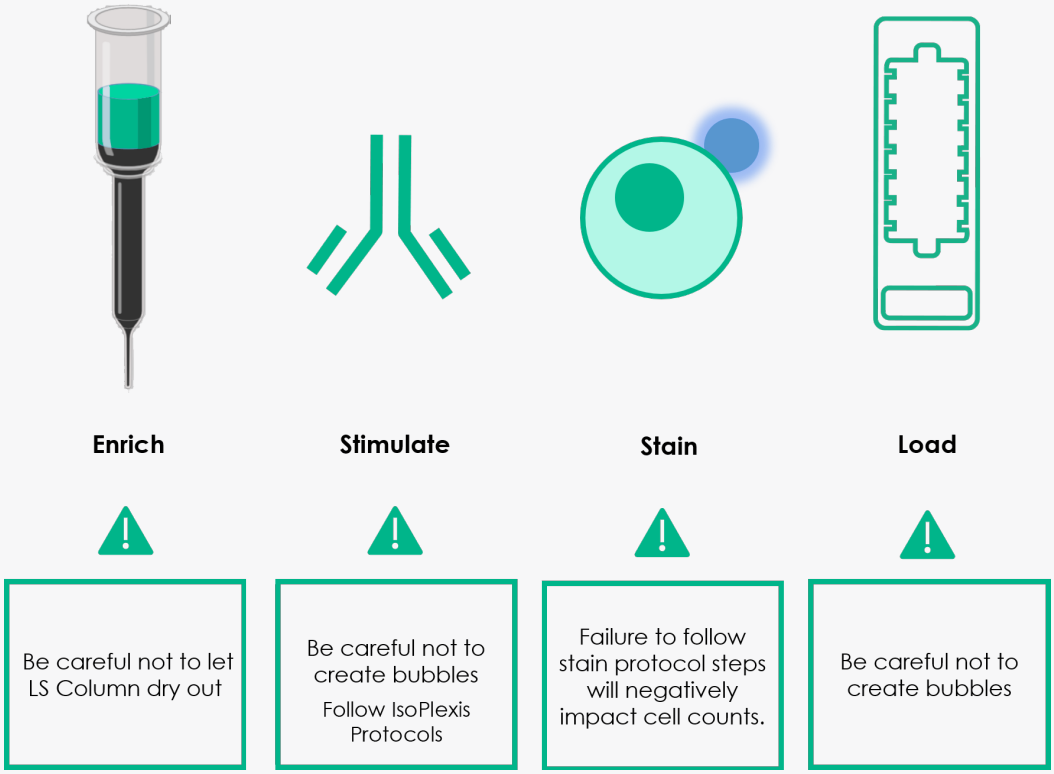

Figure 2 | Main areas of caution for each step of single-cell sample preparation

Identifying Sources of Low Cell Viability

In order to ensure optimized cell viability during the sample preparation phase of our validated protocols, it is critical to follow recommended enrichment methods. The goal of utilizing the recommended enrichment methods is to capture the phenotypes that will provide enough polyfunctional cells to compare samples.

Bead enrichment is the recommended and validated enrichment protocol for Bruker's single-cell applications. Common contributors of decreased cell viability during the enrichment and stimulation steps of sample preparation can be isolated in sample handling. During the Enrichment step, it is critical to avoid letting the LS column dry out when using the MACS bead separation kit. Be careful not to generate any bubbles while pipetting, especially during the stimulation steps. While staining, it will be critical to follow all steps and procedures ensure proper cell counts. While loading the chip it will be critical to avoid generating or pipetting bubbles into the chip. Following recommended sample handling techniques can ensure that required viable cell counts are achieved.

Utilizing Recommended Enrichment Methods.

ENRICHMENT GOAL

Capture phenotype that will provide enough polyfunctional cells to compare samples

REQUIREMENTS

- >80% viability before loading, minimum 25,000 cells per chip
- Retain recommended immune cell sub-types versus narrow multi-phenotype subsets
- Greater than 200 single cells on chip of a given phenotype, enables recognition of polyfunctional subsets

WHY RECOMMENDED PROTOCOLS EXIST

- **Single sub-type phenotypic enrichment of T cells (i.e. CD4+) is recommended to reveal differences.** The cytokine producing subsets, around 5-25% of these cells, drive correlations with in vivo biology. Multi-step phenotypic enrichment of a given subtype may miss polyfunctional sub-groups of an immune cell sample, i.e. the activity of key subtypes of CD4 or CD8 T-cells that drive correlates, simply based on cell frequency
- **Multi-step enrichment processes are to be avoided based on viability as well:** These multi-step enrichment processes additionally put greater stress on the sample and reduce overall cell counts for analysis.
- **Bead enrichment is the recommended and validated enrichment protocol for Bruker's systems:** While other enrichment methods, i.e. flow sorting, are compatible with IsoCode Technology, bead enrichment ensures higher viability when getting started with Bruker's systems. Additionally, as seen in "Sample Preparation: Technology Validation" Technology Note, many of the key in vivo correlates have been obtained with bead enrichment on Bruker's systems

Figure 3 | Enrichment Methods

Bruker's recommended enrichment methods are optimized for cell viability on Bruker's systems (Figure 3). These enrichment methods require:

- 80% viability before loading, minimum 25,000 cells per chip
- Retain recommended immune cell sub-types versus narrow multi-phenotype subsets
- Greater than 200 single cells on chip of a given phenotype, enables recognition of polyfunctional subsets

Single sub-type phenotypic enrichment of T cells (i.e. CD4) is recommended to reveal differences.

The cytokine producing subsets, around 5-25% of these cells, drive the correlative data. Multi-step phenotypic enrichment of a given subtype may miss polyfunctional sub-groups of an immune cell sample, i.e. the activity of key subtypes of CD4 or CD8 T cells that drive correlates, simply based on cell frequency.

Multi-step enrichment processes are to be avoided based on viability as well. These multi-step enrichment processes additionally put greater stress on the sample and reduce overall cell counts for analysis.

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Greater than 80% sample viability is required prior to loading on chip

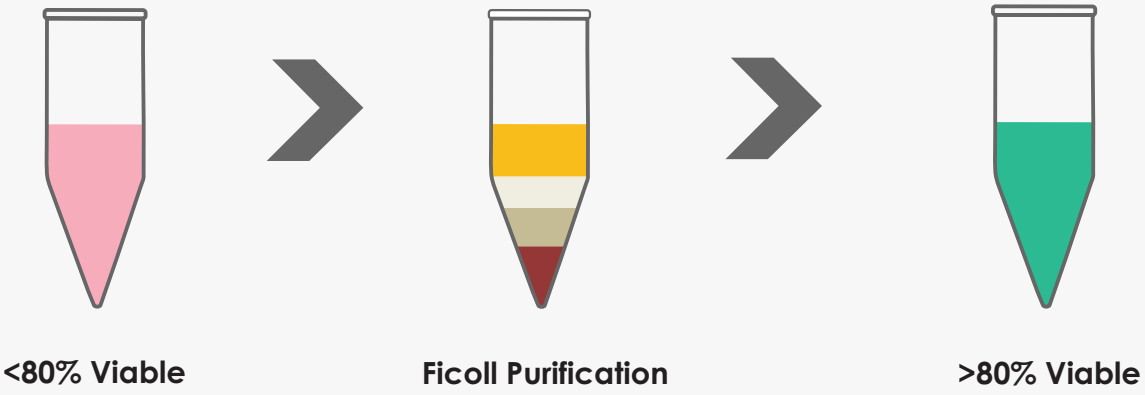

Figure 4 | If the cell viability (approximate percentage of living/viable cells in your sample) of the sample is less than 80%, it will be necessary to proceed to the dead cell depletion step using the Ficoll purification method. Once this is completed, the sample should be tested again to ensure that the sample has more than 80% viable cells.

While other enrichment methods, i.e. flow sorting, are compatible with IsoCode Technology, bead enrichment ensures higher viability when getting started with Bruker's systems. Additionally, as seen in the "Sample Preparation: Technology Validation" Technology Note, many of the key *in vivo* correlates have been obtained with bead enrichment on Bruker's systems.

Sample Viability Required Prior to Loading on Chip

Low cell viability and low sample quality is the largest single cause of variation on platform, thus it is critical to ensure that samples have greater than 80% viability prior to loading them into the IsoCode Chips (Figure 4).

If the cell viability (approximate percentage of living/viable cells in your sample) of the sample is less than 80%, it will be necessary to proceed to the dead cell depletion step using the Ficoll purification method. Once this is completed, the sample should be tested again to ensure that the sample has more than 80% viable cells.

Data Normalization Based on Sample Viability

Using IsoSpeak, researchers can easily normalize their data based on sample viability. After annotations of your sample details within the project organization, users can generate advanced visualizations with data normalized by viability. Within the 'Table Options' menu, Toggle on the 'Normalize by Viability' button, and IsoSpeak will generate all visualizations using this normalized data. This option should only be selected after following all previous recommendations within this technical note.

IsoSpeak data normalization based on sample viability

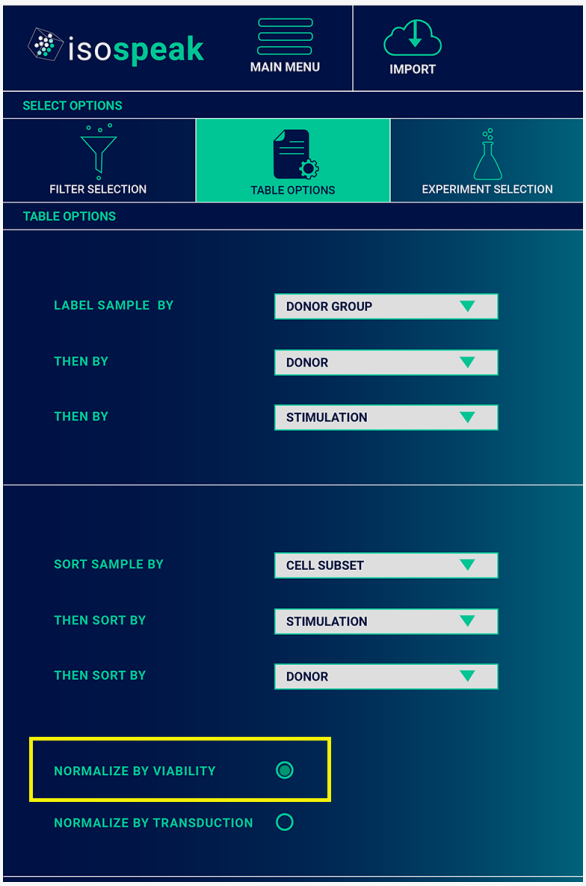

Figure 5 | IsoSpeak allows you to easily normalize your data based on viability.