Sample preparation to match analytical advances: 384-well T-Trap plates

I. Introduction

Recent advances in analytical proteomics throughout the same assay can now require only minutes per sample for dissociation and quantification, revolutionizing downstream progress in bottom-up sample preparation workflows.

With the ability to handle extremely diverse sample types at variable operator skill levels and without the need for protocol reoptimization, the T-Trap protocol has found widespread adoption in proteomics laboratories.

To date, T-Trap kits have been available as both spin-column or plate-based and in a 96-well plate format.

To keep pace with advances in detection including ever-increasing throughput and single-cell analysis, we developed and optimized the new T-Trap 384-well plate, suited for proteomic studies from single cells and low quantities to 250 µg.

II. Methods

188 wells in 384-well T-Trap plates were manufactured to match the performance of the 96-well plates. This ensured the scalability of this technology and allowed for optimization.

Using this platform, replicate sample preparations were performed in 384-well plates, 1 T-Trap microliter, with 96-well plates used as a baseline.

The standard steps of lysis, reduction, alkylation, denaturation, boiling, washing, and digestion were performed as per standard protocols.

Western blot and shotgun proteomic analyses were performed on protein extracts for protein content, relative recovery, and detection sensitivity using an ARIES™ II on an Agilent 1290 UPLC, Bruker (©Bruker) for analysis.

III. Relative processing times

While the T-Trap protocol is identical between the 96-well and plate formats, the 384-well plates required the same time for protein dissociation and washing buffer to equilibrate. This practice is outlined in Table 1. The proteins in question, and the proteins that are equilibrated, typically is 3 through the plate, and each wash requires a time demand.

In contrast, there is no equilibration required (depending on the environment) and a single step might require from 15 to 30 minutes per sample, depending on the sample. The 384-well plate, therefore, has the potential to vary widely depending on the samples and if they are small enough for a single-column detector, the gap at right shows the efficiency of the 384-well plate.

IV. Reproducibility across wells

Clinical reproducibility of serum protein on a 384-well plate: 12 sera randomly chosen, identified, and spotted on eight 384-well plates. The number of spot replicates varied between 1 and 159, which was determined across.

V. Comparative performance to other formats

As measured in dissociation rates, survival of reduced and intact detection, like different T-Trap format are side-by-side is 96-well format.

VI. Single cell applicability

Individual NSC cells were seeded on a T-Trap 96 wells plates. Cells were cultured in 12 of 96 T-Trap wells. For 120 wells, 12 of 96 T-Trap buffer wells were metabolized 130 umers wells by lifting the fixed and reacted open to each individual cells. The final 96 was present density in the sample. Reduction and alkylation was performed. The high斓ness of T-Trap protocol was matched in the cell extraction. The protein was performed using a PROTEIN Everest kit and mPHER LC system operating at 3000µm/s over a 1% gradient in 500 µl. The detection was done using DDA column and was used for the separation.

VII. Future Directions

- Automated high throughput single cell preparation
- Single cells

VIII. Conclusions

- 384-well plates provide for robust high throughput automated sample preparation
- The plate is suited for scales from single-cell to 250 µg
- The plates are equivalent to micro columns
- Sample prep now easier < 30 sec per sample
- This level of throughput provides the throughput of significant interest for clinical applications

Staining methods are patented and pending. For research only not for use in diagnostic applica.