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BCA-no-more: high throughput protein quantification directly on S-Trap plates

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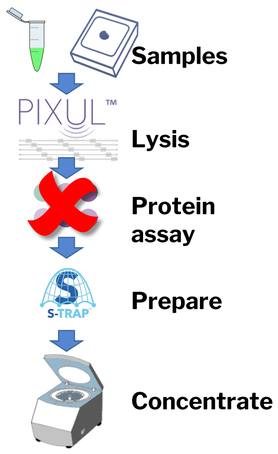
Introduction

Recent advances in the throughput of analytical proteomics, now often requiring only minutes per sample for identification and quantification, necessitate concomitant advances in bottom-up sample preparation workflows.

Specifically, the simplification and ideally elimination of any extraneous steps in a sample preparation workflow increases both throughput and robustness.

Post lysis, one of the most standard steps preceding sample preparation, is a protein concentration assay, often via BCA due to its compatibility with detergents such as SDS. However, all assays, including BCA, are not instant, are subject to interference and, if performed in a 96-well plate, edge effects.

Here, we demonstrate the new concept of direct quantification of cleaned, surface-bound protein on S-Trap 96-well plates using intrinsic protein fluorescence.

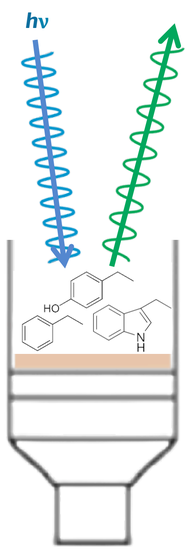


Principle

S-Trap plates and columns are specifically designed to trap protein and clean them of contaminants, such as buffers, reducing agents, detergents and other small molecules. Such small molecules frequently interfere with protein assays: detergents for Bradford, reducing agents for BCA, anything that absorbs at 280 nm for absorption, and anything fluorescent for quantification by fluorescence.

As with all solid-phase chromatography resins, protein applied to S-Trap columns loads first at the head of the column or well. As sites of affinity are occupied, the band of column loading progresses deeper into the protein trap. This surface-concentrated presentation of intrinsically fluorescent tryptophan, tyrosine and phenylalanine residues allows fluorescent protein quantification via top excitation and top emission detection. Protein quantification occurs in the exact same plate used in downstream sample processing, removing the need for a separate protein assay and the sacrifice of sample for that assay.

This approach enables the use of reagents during sample prep that would otherwise interfere with later protein assays, such as reducing agents in the solubilization of FFPE samples. This approach is a general-purpose, patent-pending technique that makes the entire range of lysis buffer reagents compatible with protein quantification.



Methods

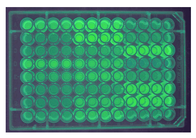
S-Trap 96-well plates were made and used as per standard processes and protocols.

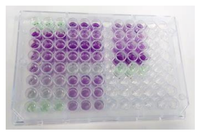
Samples of varied hydrophobicity including serum (most hydrophilic), HeLa or HEK cell lysate (both hydrophilic and hydrophobic) and rabbit brain acetone powder (most hydrophobic) were bound onto plates and washed as per standard protocols.

Standard BSA curves were also loaded.

Protein fluorescence in both a wet and dry state was measured with an excitation between 269 and 280 nm and an emission of 325 to 475 nm using a Tecan Sparc plate reader in top-read excitation and emission mode.

Protein concentrations determined via protein fluorescence were compared to BCA for limit of detection, reproducibility, and dynamic range.





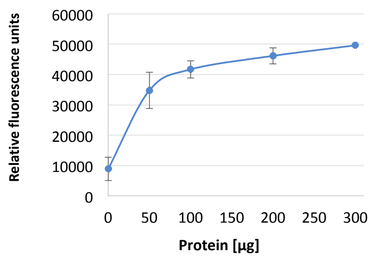
Method Parameters

The optimal z-position (2100 µm) for fluorescence protein concentration quantification on the S-Trap 96-well plate using a Tecan Sparc was experimentally determined on bound protein.

When provided with digestion buffer (e.g. 50 mM TEAB at pH 7.5), fluorescence tracked with protein load at 277 nm excitation and 350 nm emission; fluorescence was quenched as buffer pH became acidic, eventually reaching near-background levels

As expected, given how the S-Trap (and other solid phases) load, there was a reasonably linear response up to ~100 µg per well. After this, the linearity of response dropped significantly, a phenomenon attribute to fluorescent moieties binding deeper in the trap, which is not UV transparent.

The direct-determination method afforded protein quantification in a significantly reduced time compared to BCA assays with dynamic range and sensitivity compatible with standard bottom-up and top-down proteomics workflows.

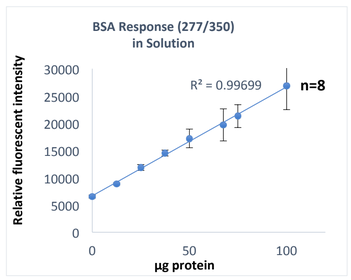


Equivalence to In-Solution Fluorescence

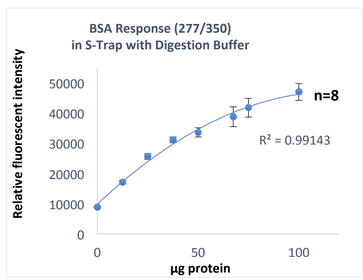
The S-Trap sample preparation workflow successfully removed matrix contaminants prior to protein concentration determination without the need for additional steps.

Such on-plate protein concentration determination lends itself directly to deployment in high-throughput clinical settings using automated fluid handlers.

Here we demonstrate the equivalence of this technique on-plate compared to in-solution fluorescent measurements on the same sample: both curve fits and CVs are comparable.



µg protein	%CV
0.0	4.6%
12.5	2.3%
25.0	4.7%
37.5	3.8%
50.0	9.9%
67.5	15.0%
75.0	10.0%
100.0	16.2%
Average	**8.3%**

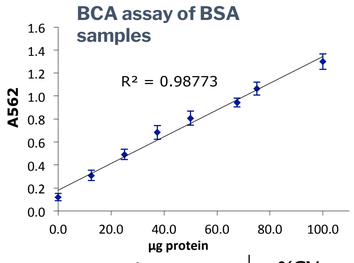


µg protein	%CV
0.0	1.6%
12.5	2.9%
25.0	3.7%
37.5	3.1%
50.0	4.6%
67.5	8.6%
75.0	7.5%
100.0	5.9%
Average	**4.7%**

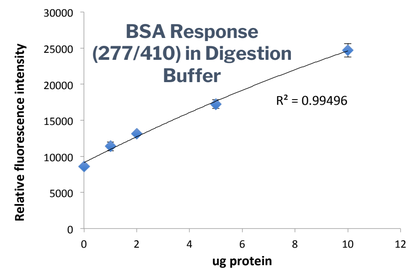
Equivalence to BSA

Protein quantification via an on-plate fluorescence reading was of comparable accuracy to in-solution BCA of the same sample without necessitating sample loss, incubation or even further manipulation than loading onto the S-Trap 96-well plate.

Background fluorescence readings of protein-free trapping matrix decreased with increasing emission wavelength. At 277 nm excitation and 410 emission, it was possible to detect as little as 1 µg of protein. Such small quantities are virtually impossible to quantify by colorimetric assays yet are frequently encountered in laser capture micro-dissection.



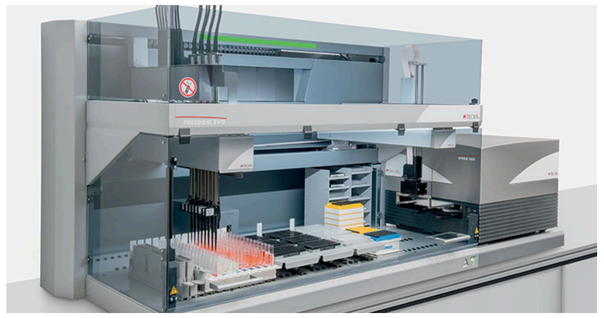
µg protein	%CV
0.0	6.8%
12.5	5.8%
25.0	3.8%
37.5	6.1%
50.0	6.0%
67.5	4.6%
75.0	4.5%
100.0	3.2%
Average	**5.1%**



µg protein	%CV
0	1.7%
1	5.4%
2	1.8%
5	3.5%
10	3.8%
Average	**3.3%**

Conclusion

This patent-pending "BCA-no-more" direct determination of protein concentration with intrinsic clean-up removes the need for additional costly protein quantification assays and fits directly in automated sample preparation workflows.
A typical BCA kit costs ~$US200 – $US300 and might take an experienced operator 30 – 60 min, depending on the number of samples, replicates, etc. Here, we remove the need for both an additional kit and sample sacrifice, extend sensitivity and quantification to the single µg level out of any kind of lysis buffer and do so in a format amenable to high-throughput automation.



BCA	S-Trap "BCA no more"
$200-$300 per kit	No extra kit required
30-60 minutes	Just the read time of the plate
Minimum sample amount (LLOD)	No need to sacrifice precious sample
Must avoid high concentrations of reducing agents	Suitable for harsh lysis buffers including those for FFPE

ProtiFi technologies are patented and patent-pending.