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Recommendations for Various Starting Volumes

Sample Type	S-Trap™ 1x Lysis Solution (µL)	S-Trap™ Solution 1, 2x Lysis Solution (µL)	S-Trap™ Solution 2, Reductant (µL)	S-Trap™ Solution 3, Alkylator (µL)	S-Trap™ Solution 4, Acidifier (µL)	S-Trap™ Solution 6, Binding/ Wash Solution (µL)
Reagent:Sample Ratio (v/v)	1:1	1:1	1:23	1:23	1:10	6:1
Pelleted Cells	23	-	1	1	2.5	165
Protein Pellet	23	-	1	1	2.5	165
11.5 µL Liquid	-	11.5	1	1	2.5	165
15 µL Liquid	-	15	1.3	1.3	3.3	215
30 µL Liquid	-	30	2.6	2.6	6.5	430
100 µL Liquid	-	100	8.7	8.7	21.7	1,435

The procedure can be scaled to different initial sample volumes by keeping all reagent volume ratios the same.

There are a few key points to keep in mind:

1. If the protein sample does not contain SDS, either add the same volume of S-Trap™ Solution 1, 2x Lysis Solution, add dry SDS, or add SDS stock to a minimum of 2% SDS.
2. The amount of S-Trap™ Solution 2, Reductant and S-Trap™ Solution 3, Alkylator to add is always 1/23 of the initial sample volume (e.g. for 23 µL, add 1 µL). The final concentration of the Reductant is 5 mM. The final concentration of the Alkylator is 20 mM.
3. The amount of S-Trap™ Solution 4, Acidifier to add is always 1/10 of the initial sample volume, plus the added volumes of TCEP and MMTS (e.g. for 25 µL, add 2.5 µL). The final concentration of the Acidifier is 1.2% for S-Trap™ Mini Spin Columns and Midi Spin Columns and 2.75% for S-Trap™ Micro Spin Columns and 96-well Plates.
4. The amount of S-Trap™ Solution 6, Binding/Wash Solution to add is 6x the total volume of sample (initial volume plus other reagents, e.g. for (23 µL sample + 1 µL Reductant + 1 µL Alkylator + 2.5 µL Acidifier) x 6 = 165 µL, add 165 µL of Binding/Wash Solution). Due to reservoir capacity, the column might need to be loaded multiple times after the addition of Binding/Wash Solution.

Consumable Type	Reservoir Capacity (µL)
Micro Spin Columns	275
Mini Spin Columns	600
96-well Micro Plate	400
96-well Mini Plate (S-Trap™ and Turbo™)	450