

## Recommendations for Various Starting Volumes

| Sample Type                       | 1x Lysis Solution (μL) | 2x Lysis Solution (μL) | Reductant (μL) | Alkylator (μL) | Acidifier (μL) | Binding/Wash Solution (μL) |
|-----------------------------------|------------------------|------------------------|----------------|----------------|----------------|----------------------------|
| <b>Reagent:Sample Ratio (v/v)</b> | 1:1                    | 1:1                    | 1:23           | 1:23           | 1:10           | 6:1                        |
| <b>Pelleted Cells</b>             | 23                     | -                      | 1              | 1              | 2.5            | 165                        |
| <b>Protein Pellet</b>             | 23                     | -                      | 1              | 1              | 2.5            | 165                        |
| <b>11.5 μL Liquid</b>             | -                      | 11.5                   | 1              | 1              | 2.5            | 165                        |
| <b>15 μL Liquid</b>               | -                      | 15                     | 1.3            | 1.3            | 3.3            | 215                        |
| <b>30 μL Liquid</b>               | -                      | 30                     | 2.6            | 2.6            | 6.5            | 430                        |
| <b>100 μL Liquid</b>              | -                      | 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 2x Lysis Solution, add dry SDS, or add SDS stock to a minimum of 2% SDS.
2. The amount of Reductant and 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 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 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) |
|---------------------------------------|-------------------------|
| <b>Micro Columns</b>                  | 275                     |
| <b>Mini Columns</b>                   | 600                     |
| <b>Standard Micro Plates</b>          | 400                     |
| <b>Turbo Micro Plates</b>             | 450                     |
| <b>Standard and Turbo Mini Plates</b> | 450                     |