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| **IN SOLUTION DIGEST - CHECKLIST** | | | |
| Customer Name: | | Number of samples: | Date: |
|  | **Reagent** | **Reagent preparation** | Checklist: |
| **1** | 50 mM Triethylammonium bicarbonate (TEAB) | Mix 50 µl of TEAB 1M stock with 950 µl of milli Q water |  |
| **2** | 100 mM Tris  (2-carboxyethyl) phosphine (TCEP) | Mix 2 µl of 500 mM TCEP with 8 µl of 50 mM TEAB |  |
| **3** | 100 mM S- Methyl  Methanethiosulphonate (MMTS) | Mix 1 µl of MMTS with 99 µl of 2-propanol |  |
| **4** | 20 µg/µl Trypsin | Reconstitute by adding 20 µl of milli Q water to the trypsin vial containing 20 µg of trypsin |  |
| **Stepss** | | **Procedure** | Checklist: |
| **1** | Volume adjustment | If the volume is more than 10 µl, reduce volume in speed vac to approximately 10 µl. Adjust the sample volume to 10 µl using 50 mM TEAB if necessary. |  |
| **2** | Reduction: TCEP | Add 1 µl 50 mM TCEP prepared in 50 mM TEAB. Incubate 1 hour at 60°C and allow to cool to RT |  |
| **3** | Alkylation: MMTS | Add 1 µl of 100 mM MMTS prepared in 2-propanol. Incubate 15 min at RT. |  |
| **4** | Volume adjustment | Adjust sample volume to 95 µl using 50 mM TEAB. |  |
| **5** | Trypsination | Add Trypsin to the sample at a 1:20 ratio (i.e. 5µg/100µg). Vortex & parafilm tubes to prevent evaporation. Incubate at 370C for 18 hours |  |
| **6** | Extraction | Speed vac samples to dryness & resuspend in 30 µl of 2% acetonitrile/water; 0.1 % Formic acid. Samples are ready for cleanup. |  |
| Deviations: | | | |
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