|  |
| --- |
| **IN GEL DIGEST - CHECKLIST** |
| Customer Name: | Number of samples: | Date:  |
|  | **Reagent** | **Reagent preparation** | Checklist: |
| **1** | 1 M Ammonium bicarbonate (AmBic) | Dissolve 0.079g AmBic in 1 ml milli Q water |  |
| **2** | 200mM Ammonium bicarbonate (AmBic) | Mix 200 µl of 1 M AmBic with 800 µl of milli Q water |  |
| **3** | 100 mM AmBic | Mix 100 µl of 1 M AmBic with 900 µl of milli Q water |  |
| **4** | Destaining solution | Mix 500 µl of 200 mM AmBic with 500 µl 100% acetonitrile (ACN) to give a final concentration of 100 mM AmBic and 50 % ACN in 1 ml |  |
| **5** | 5 mM Tris(2-carboxyethyl) phosphine (TCEP) | Mix 10 µL of 500 mM stock with 990 µl of 100 mM AmBic |  |
| **6** | 10 mM S- MethylMethanethiosulphonate (MMTS) | Mix 10 µL of 500 mM stock with 990 µl of 100 mM AmBic |  |
| **7** | 0.02 µg/ µl Trypsin | Reconstitute by adding 1 ml 100 mM AmBic to the trypsin vial |  |
| **Steps** | **Procedure** | Checklist: |
| **1** | Destain | Add destain solution to cover gel pieces. Refresh multiple times as the dye release from the gel. |  |
| **2** | Dehydrate: Acetonitrile | Add sufficient to cover gel pieces. Remove after gel turns opaque/white. Repeat if necessary. |  |
| **3** | Reduction: TCEP | Add sufficient volume to cover gel pieces. Leave at room temp for 1 hour and discard supernatant. |  |
| **4** | Dehydrate: Acetonitrile | Add sufficient to cover gel pieces. Remove after gel turns opaque/white. Repeat if necessary. |  |
| **5** | Wash: Ammonium bicarbonate | Add sufficient volume to cover gel pieces. Leave 15 min or until gel pieces are resuspended. Discard supernatant. |  |
| **6** | Dehydrate: Acetonitrile | Add sufficient to cover gel pieces. Discard after gel turns opaque/white. Repeat if necessary. |  |
| **7** | Alkylation: MMTS | Add sufficient volume to cover gel pieces. Leave at room temp for 30 min in a dark area. Discard supernatant |  |
| **8** | Dehydrate: Acetonitrile | Add sufficient to cover gel pieces. Remove after gel turns opaque/white. Repeat if necessary. |  |
| **9** | Wash: Ammonium bicarbonate | Add sufficient volume to cover gel pieces. Leave 15 min or until gel pieces are resuspended. Remove |  |
| **10** | Dehydrate: Acetonitrile | Add sufficient to cover gel pieces. Remove after gel turns opaque/white. Repeat if necessary. |  |
| **11** | Desiccate (Speedy vac) | Place samples in a desiccator for 3-5 minutes to dry |  |
| **12** | Trypsination | Add sufficient trypsin to cover gel pieces. Leave on ice for 1 hours. Top up with ammonium bicarbonate & incubate 18 hours at 370C |  |
| **13** | Extraction | Add an equal volume of water as the reaction mixture that’s currently on the gel pieces & leave at RT for 1 hour. Transfer supernatant to an empty 1.5 mL tube (extract 1). Add 50 % Acetonitrile and leave at RT for 1 hour. Remove the supernatant & combine with extract 1. Speed vac supernatant to dryness. Store samples at -200C or resuspend in 30 µl of 2% acetonitrile/water; 0.1 % FA. Samples are ready for cleanup. |  |
| Deviations:  |
|  |