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Trypsin Digestion

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P212121

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Protocol status: Working

Created: October 23, 2014

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Abstract

How to preform a trypsin digestion

Materials

MATERIALS

 TCEP HCl **P212121 Catalog #SV-TCEP**

 DTT **P212121 Catalog #SV-DTT**

 Ammonium bicarbonate **P212121 Catalog #CI-01682**

 Urea **P212121 Catalog #RP-U20200**

 Tris **P212121 Catalog #RP-T60040**

 Iodoacetamide **P212121 Catalog #AK-U470**

STEP MATERIALS

 Ammonium bicarbonate **P212121 Catalog #CI-01682**

 Tris **P212121 Catalog #RP-T60040**

 TCEP HCl **P212121 Catalog #SV-TCEP**

 Iodoacetamide **P212121 Catalog #AK-U470**

 DTT **P212121 Catalog #SV-DTT**

 Methanol **P212121 Catalog #PA-33900HPLCCS4L**

 Trypsin **P212121 Catalog #RP-T70010**

 Ammonium bicarbonate **P212121 Catalog #CI-01682**

 Tris **P212121 Catalog #RP-T60040**

 TCEP HCl **P212121 Catalog #SV-TCEP**

 Iodoacetamide **P212121 Catalog #AK-U470**

 DTT **P212121 Catalog #SV-DTT**

 Methanol **P212121 Catalog #PA-33900HPLCCS4L**

 Trypsin **P212121 Catalog #RP-T70010**



Protocol materials

☒ Methanol **P212121 Catalog #PA-33900HPLCCS4L**

☒ Trypsin **P212121 Catalog #RP-T70010**

☒ Ammonium bicarbonate **P212121 Catalog #CI-01682**

☒ Tris **P212121 Catalog #RP-T60040**

☒ TCEP HCl **P212121 Catalog #SV-TCEP**

☒ Urea **P212121 Catalog #RP-U20200**

☒ Iodoacetamide **P212121 Catalog #AK-U470**

☒ DTT **P212121 Catalog #SV-DTT**



- 1 Make 6M Urea solution by adding 108.11 mg of Urea to 300 μ L of 50mM ammonium bicarbonate (NH_4HCO_3) containing protein.
 00:04:00
 Ammonium bicarbonate **P212121 Catalog #CI-01682**
- 2 Add 20 μ L of 1.5 M Tris pH 8.8.
 00:02:00
 Tris **P212121 Catalog #RP-T60040**
- 3 Add 7.5 μ L of 200mM TCEP and incubate for 1 hour at 37°C.
 01:00:00
 TCEP HCl **P212121 Catalog #SV-TCEP**
- 4 Add 60 μ L of 200mM iodoacetamide and vortex. Incubate for one hour at room temperature in a dark area.
 01:02:00
 Iodoacetamide **P212121 Catalog #AK-U470**
- 5 Add 60 μ L of 200mM DTT and vortex. Incubate for one hour at room temperature.
 01:02:00
 DTT **P212121 Catalog #SV-DTT**
- 6 Aliquot 150 μ L each into three 1.5 mL centrifuge tubes.
 00:05:00
- 7 Add 800 μ L of 25mM ammonium bicarbonate to each tube to dilute the urea.
 00:03:00
- 8 Add 200 μ L of MeOH to each tube.
 00:03:00
 Methanol **P212121 Catalog #PA-33900HPLCCS4L**
- 9 Add Trypsin to protein at a ratio of 50 protein to 1 trypsin and incubate overnight at room temperature. Speedvac until dry.
 24:05:00
 Trypsin **P212121 Catalog #RP-T70010**
- 10 Add 200 μ L of molecular biology grade water to tubes three times and speedvac to reduce the ammonium bicarbonate. Store at -80°C.



00:03:00