

# Trypsin Digestion

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## Abstract

How to preform a trypsin digestion

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## Materials

- TCEP HCl [SV-TCEP](#) by [P212121](#)
- DTT [SV-DTT](#) by [P212121](#)
- Ammonium bicarbonate [CI-01682](#) by [P212121](#)
- Urea [RP-U20200](#) by [P212121](#)
- Tris [RP-T60040](#) by [P212121](#)
- Iodoacetamide [AK-U470](#) by [P212121](#)

## Protocol

### Step 1.

Make 6M Urea solution by adding 108.11 mg of Urea to 300  $\mu$ L of 50mM ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) containing protein.



#### REAGENTS



Ammonium bicarbonate [CI-01682](#) by [P212121](#)



#### DURATION

00:04:00

### Step 2.

Add 20  $\mu$ L of 1.5 M Tris pH 8.8.



#### REAGENTS



Tris [RP-T60040](#) by [P212121](#)



#### DURATION

00:02:00

### Step 3.

Add 7.5  $\mu$ L of 200mM TCEP and incubate for 1 hour at 37°C.



#### REAGENTS



TCEP HCl [SV-TCEP](#) by [P212121](#)



#### DURATION

01:00:00

#### Step 4.

Add 60 µL of 200mM iodoacetamide and vortex. Incubate for one hour at room temperature in a dark area.



##### REAGENTS



Iodoacetamide [AK-U470](#) by [P212121](#)



##### DURATION

01:02:00

#### Step 5.

Add 60 µL of 200mM DTT and vortex. Incubate for one hour at room temperature.



##### REAGENTS



DTT [SV-DTT](#) by [P212121](#)



##### DURATION

01:02:00

#### Step 6.

Aliquot 150 µL each into three 1.5 mL centrifuge tubes.



##### DURATION

00:05:00

#### Step 7.

Add 800 µL of 25mM ammonium bicarbonate to each tube to dilute the urea.



##### DURATION

00:03:00

#### Step 8.

Add 200 µL of MeOH to each tube.



##### REAGENTS



Methanol [PA-33900HPLCCS4L](#) by [P212121](#)



##### DURATION

00:03:00

#### Step 9.

Add Trypsin to protein at a ratio of 50 protein to 1 trypsin and incubate overnight at room temperature. Speedvac until dry.



##### REAGENTS



Trypsin [RP-T70010](#) by [P212121](#)



##### DURATION

24:05:00

#### Step 10.

Add 200 µL of molecular biology grade water to tubes three times and speedvac to reduce the ammonium bicarbonate. Store at -80°C.



##### DURATION

00:03:00