PROTOCOL: Trypsin Digest (TRY)

Purpose

To digest proteins into peptides. Trypsin cuts at Arginine and Lysine, but due to the previous propionylation of lysine residues, the resulting peptides should all have C-terminal arginines.

Preparation

1. Thaw 10 Promega trypsin vials (20ug) {TRY-M03} on ice
2. Prepare MIX01 (50mM Ammonium Bicarbonate, pH 8.0)
3. Prepare MIX02 (0.1ug/uL Trypsin)
4. If shaker on LT-BRAVO is at room temperature, set shaker to 37℃

Materials

- HPLC-grade water {TRY-M01} [JT Baker, 4218-03]
- Ammonium Bicarbonate {TRY-M02} [Sigma-Aldrich, A6141-500G]
- Promega Trypsin (20ug) {TRY-M03} [Promega, V5113]
- 500uL V-bottom plate {TRY-M04} [VWR, 89005-016]
- 96-Well skirted PCR plate {TRY-M05} [Bio-Rad, MSP-9601]
- 1-Well Low Profile Reagent Reservoir {TRY-M06} [Axygen, RES-SW1-LP]
- Agilent 96LT-250uL Tips {TRY-M07} [Agilent, 19477.002]
- Axygen -80°C Rated Foil Seal {TRY-M08} [Axygen, PCR-AS-200]
- Breath-EASIER seal {TRY-M09} [Diversified Biotech, BERM-2000]

Assets

- Agilent LT-Bravo Automated Liquid Handling Platform with VWorks4 {TRY-A01}
- Thermo Scientific Savant SC210A Concentrator {TRY-A02}

Reagent Mixes

ID	Name	Step	Composition	Volume/Well	Use
MIX01	50mM Ammonium Bicarbonate, pH 8.0	**TRY**	3.9528mg/mL ammonium bicarbonate {TRY-M02} in HPLC-grade water {TRY-M01}	~25mL	Resuspend dried proteins and keep digestion reaction at pH 8.0.
MIX02	0.1ug/uL Trypsin	**TRY**	0.1ug/uL Promega trypsin {TRY-M03} in 50mM Ammonium Bicarbonate {MIX01}	20uL	Digestion reagent.

Mix Preps and Mini-worksheets:

MIX01 – 50mM Ammonium Bicarbonate, pH 8.0

1. Weigh out at least 39.528mg of ammonium bicarbonate to make at least 10 mL
2. Calculate amount of water to add in mL by dividing amount weighed out by 3.9528
   - ❏ Amount weighed: _____ mg
   - ❏ Divide by: 3.9528
   - ❏ Water to add _____ mL.

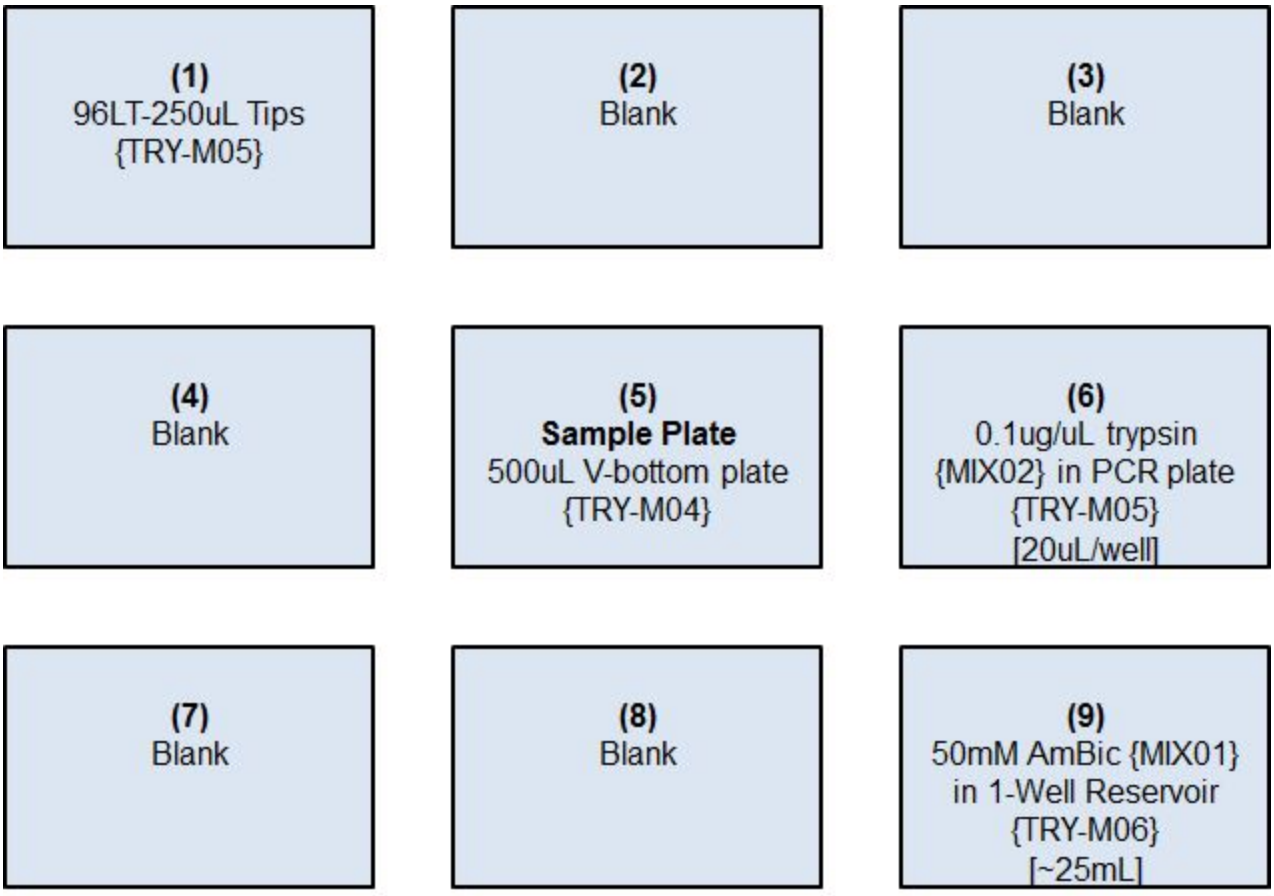
MIX02 – 0.1ug/uL Trypsin

1. Thaw 10, 20ug vials of Promega trypsin {TRY-M03} on ice for 96 samples. ❏
2. Add 150uL of cold 50mM Ammonium bicarbonate {MIX01} to each vial and vortex to mix. ❏
3. Keep on ice until use. ❏

NB: Promega trypsin vials (20ug) {TRY-M03} contain 20ug of trypsin in 50uL of solvent. The starting concentration of the trypsin is 0.4ug/uL.

Procedure

1. Fill a 1-well reagent reservoir {TRY-M06} with approximately 25mL of 50mM Ammonium Bicarbonate, pH 8.0 {MIX01}.
2. Fill each well of a 96-Well skirted PCR plate {TRY-M05} with 20uL of 0.1ug/uL trypsin {MIX02}.
   2.1. Keep on ice.
3. On the LT-BRAVO Marvin {A01} load the device file “Bravo with Heated Shakers.dev”. This file is located at C:\\VWorks Workspace\Device Files\.
4. In the “Devices” page, click on “Agilent LT-BRAVO” and then “Marvin”. Select “Initialize all devices”.
5. Open the protocol file “TrypsinDigest_1ugEnzyme.pro”. This file is located at · C:\\VWorks Workspace\Protocol Files\CF\Histones\.
6. Assemble the deck of the LT-BRAVO according to the following layout:



7. On the LT-BRAVO, toggle to “Simulation is on” at the top of the screen from “Simulation is off”.
   7.1. Press Start and the Run Configuration Wizard will pop up. Press Finish.
   7.2. A pop up entitled “Set Initial Values for Variables” will appear. Set the number of “Columns” to the appropriate amount of sample columns and press ok.
   7.3. The simulation will run and provide feedback on any warnings or errors that the protocol may encounter. If there are any unknown errors that come up, notify the key LT-BRAVO user and obtain help.
8. On the LT-BRAVO, toggle back to “Simulation is off”. Follow steps 7.1 to 7.3 to run the protocol.

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NB: The protocol will pause after the addition of 0.1ug/uL trypsin.
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9. After the protocol has paused, place a foil seal {TRY-M08} on the sample plate and replace the plate in position 4 on the LT-BRAVO deck.
   9.1. Press GO.
   9.2. The LT-BRAVO will move the plate to the shaker where it will incubate at 37°C for 15 hours (overnight) with shaking at 800rpm.
10. Clear the deck, cover the sample plate with a breathable seal {TRY-M09} and then a foil seal {TRY-M08}, and freeze at -80°C
11. Remove the foil seal and speedvac samples to dryness.
12. Continue on to **Secondary Propionylation (K2)**.