

Protocol: Protein or Peptide Neutralization with EndoTreat™

Materials

Kit 1 vial Sample Buffer
1 vial Protease Mix
(1 kit can be used for at least 20 reactions)

User-provided

- Endotoxin-free glass tubes, pipette tips, etc.
- Water bath
- LAL or recombinant Factor C assay kit

EndoTreat Preparation and Storage

1. Upon receipt, please store EndoTreat™ kit at 4°C. The EndoTreat™ kit is stable for 2 years when properly stored, prior solubilizing the Protease Mix.
2. Prior starting an experiment, add 1 mL of Sample Buffer into the Protease Mix vial to form Protease Mix Solution.
3. Mix the solution vigorously by vortexing for 5 minutes.
4. Ensure complete solubilization by visual inspection.
5. Protease Mix Solution and Sample Buffer should be stored at 4°C after use.
6. After the preparation of Protease Mix Solution, EndoTreat™ kit is stable for 3 months when properly stored.

Procedure

1. Prepare sample by diluting to a concentration of 0.1 to 1.0 mg/mL with Sample Buffer. Sample Buffer should account for at least 90% of the sample volume for proper protease activity. If dilution is not possible, use diluted HCl to lower the pH of the sample below 4.5.
2. Remove an aliquot of 270 µL and transfer to a sterile, endotoxin-free borosilicate glass tube.
3. Add 30 µL of Protease Mix into the glass tube and mix the content by vortexing for 10 seconds. A control sample containing 30 µL of Sample Buffer instead of Protease Mix should be included with each set of reactions to determine baseline endotoxin content without digestion.
4. Cover tubes with Parafilm.
5. Incubate tubes in a 37°C water bath. Most proteins and peptides show maximum endotoxin activity after 60 minutes of treatment. Some samples may require longer incubation. To verify complete sample digestion, polyacrylamide gel electrophoresis should be performed on digested samples.
6. After digestion, dilute samples 1:100 with endotoxin-free water. Dilution of only 1:10 is possible but should include proper validation that enzyme or digestion products do not interfere with the endotoxin detection assay at this concentration.
7. Test with LAL or recombinant Factor C assay. Samples treated with EndoTreat™ should be tested both with and without a positive product control (PPC). Samples of Sample Buffer and Protease Mix at equivalent concentrations should be tested alongside all samples as control.

Order Information

Order Cat. #: SB-005-001
EndoTreat Sample Treatment Kit
Price: \$ 299 USD

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Send order to: cweaver@stablebiologics.com