

Soybean Trypsin Inhibitor (STI) Preparation and Use

Cell Culture Trypsin Inhibition

Introduction

Trypsin inhibitors are serine protease inhibitors that specifically regulate the biological activity of trypsin, an enzyme involved in the digestion of proteins. Natural forms of trypsin inhibitors, such as soybean trypsin inhibitor, act as competitive substrates for serine proteases, binding the protease and causing it to become inactive. Soybean trypsin inhibitors act as a feeding deterrent in insects and have been used in the development of insect resistant transgenic plants. It is also widely used in cell culture to inhibit tryptic activity during cell disassociation in order to prevent cell damage or death.

Before use, check the subcultivation specifications of the cells which are being used in culture. STI typically forms a 1:1 stoichiometric complex with trypsin but some cell lines have historically required additional STI to dissociate properly. Soybean Trypsin Inhibitor is a reversible competitive inhibitor of trypsin and trypsin-like proteases. Chymotrypsin is only slightly inhibited by Soybean Trypsin Inhibitor.

Materials

- [Soybean Trypsin Inhibitor Stock Solution – 5 mg/ml](#), prepared in PBS (not water)
- [PBS Solution \(PBS Tablets, GoldBio Catalog # P-271\)](#)

Method

Prepare an appropriate concentration of STI working solution to match the concentration of trypsin used in the cell culture. Three common concentrations are detailed below (for other concentrations of trypsin, adjust STI working solution concentration accordingly):

1. Prepare 2.5 mg/ml working solution
 - a. Bring STI Stock Solution (5 mg/ml) to room temperature.
 - b. Mix 20 ml of STI Stock Solution in 20 ml PBS.
 - i. The final working concentration is 2.5 mg/ml STI.
 - ii. 1 ml working solution will inhibit ~2.5 mg trypsin or 1 ml of 0.25% trypsin solution.
2. Prepare 1 mg/ml Solution
 - a. Bring STI Stock Solution (5 mg/ml) to room temperature.
 - b. Mix 10 ml of STI Stock Solution in 40 ml PBS.
 - i. The final working concentration is 1.0 mg/ml STI.

- ii. 1 ml working solution will inhibit ~1.0 mg trypsin or 1 ml of 0.1% trypsin solution.
3. Prepare 0.5 mg/ml Solution
 - a. Bring STI Stock Solution (5 mg/ml) to room temperature.
 - b. Mix 10 ml of STI Stock Solution in 90 ml PBS
 - i. The final working concentration is 0.5 mg/ml STI.
 - ii. 1 ml working solution will inhibit ~0.5 mg trypsin or 1 ml of 0.05% trypsin solution.

Note: STI working stock solutions should be kept at 2-8°C and is stable for 30 days.

Trypsin Inhibition

1. Follow standard instructions for trypsinization of cells.
2. After trypsinization, resuspend cells in an equal concentration and equal volume (1:1) of STI working solution.
3. Centrifuge cell suspension at 125 x g for 5 minutes.
4. A cell pellet should form. Remove as much of the trypsin inhibitor solution as possible and resuspend the pellet in serum free media. Cells may be cultured as desired.

Calculations

Soybean Trypsin Inhibitor Unit Definition:

One unit will produce a ΔA_{253} of 0.001 per minute at pH 7.6 and 25°C using BAEE as a substrate.