



Soybean Trypsin Inhibitor

30-2104™

Description

The use of Soybean Trypsin Inhibitor (SBTI) provides a serum-free option with which to inactivate trypsin when subculturing adherent cells. Following dissociation, trypsin is typically inactivated by adding a serum-containing medium. A serum-free option is preferred for certain applications; e.g. when growing cells under serum-free conditions.

SBTI is a single polypeptide chain cross-linked by two disulfide bonds that forms a stable, enzymatically inactive complex with trypsin. SBTI is a reversible competitive inhibitor of trypsin and other trypsin-like proteases. It forms a 1:1 stoichiometric complex with trypsin; upon formation of this complex, trypsin may cleave a single arginine-isoleucine bond in the inhibitor forming covalent bimolecular complex of inhibitor and trypsin.

It acts as an inhibitor only when it is in its native state. Addition of increasing amounts of SBTI to a solution of trypsin decreases the proteolytic activity of the trypsin in direct proportion to the amount of inhibitor added. This reaction is practically instantaneous and is independent, within a wide range, of the pH of the solution. Chymotrypsin is only slightly inhibited by SBTI as this interaction results in the formation of a reversibly dissociable compound; SBTI has no effect on the activity of pepsin. This product has applications for cell culture, cell growth, and viability.

Volume: 20 mL

Storage Conditions

Product format: Frozen

Storage conditions: -20°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

Biosafety Information

ATCC determined that a biosafety level is not applicable to this material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to complete your own risk assessment and understand any potential hazards associated with the material per your organization's policies and procedures and any other applicable regulations as enforced by your local or national agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

Preparation of the Soybean Trypsin Inhibitor Solution

Prior to thawing the Soybean Trypsin Inhibitor Solution, check the subcultivation recommendations for the cells you are planning to dissociate. While Soybean Trypsin Inhibitor Solution forms a 1:1 stoichiometric complex with trypsin, some cell cultures have been historically dissociated using a concentration of soybean trypsin inhibitor that is twice the concentration of the trypsin. Based on the conditions suitable for the cell line, choose one of the following methods of preparation:

Preparing a 0.05% Soybean Trypsin Inhibitor Solution:

Bring the Soybean Trypsin Inhibitor Solution to room temperature. Aseptically dilute the thawed SBTI 1:10 in D-PBS (e.g., 20 mL SBTI into 180 mL D-PBS). The final working concentration is 0.5 mg SBTI /mL. After dilution, 1 mL of SBTI working solution will inhibit 0.5 mg trypsin or 1 mL of 0.05% trypsin solution (specific activity is 10,000 BAEE units/mg protein).

Preparing a 0.1% Soybean Trypsin Inhibitor Solution:

Bring the Soybean Trypsin Inhibitor Solution to room temperature. Aseptically dilute the thawed SBTI 1:5 in D-PBS (e.g., 20 mL SBTI into 80 mL D-PBS). The final working concentration is 1.0 mg SBTI /mL. After dilution, 1 mL of SBTI working solution will inhibit 1.0 mg trypsin or 1 mL of 0.1% trypsin solution (specific activity is 10,000 BAEE units/mg protein).

Preparing a 0.25% Soybean Trypsin Inhibitor Solution:

Bring the Soybean Trypsin Inhibitor Solution to room temperature. Aseptically dilute the thawed SBTI 1:2 in D-PBS (e.g., 20 mL SBTI into 20 mL D-PBS). The final working concentration is 2.5 mg SBTI /mL. After dilution, 1 mL of SBTI working solution will inhibit 2.5 mg trypsin or 1 mL of 0.25% trypsin solution (specific activity is 10,000 BAEE units/mg protein).

The working solution of SBTI should be stored at 2°C to 8°C (do not freeze). When stored under these conditions, the working solution of SBTI is stable for 60 days.

General Subculture Procedure using SBTI

Each type of cell or cell line responds to trypsin in a unique manner. For optimum results, continuously observe the cells during the dissociation process to prevent damage. For cell-specific information, please refer to the product sheet supplied with the cells or cell line.

1. Bring the following components to room temperature before use:
 - a. D-PBS
 - b. Trypsin or trypsin-EDTA

c. Prepared Soybean Trypsin Inhibitor

2. Warm the complete growth medium to 37°C prior to use with the cells.
3. For each vessel, carefully aspirate the spent media without disturbing the monolayer. If the cell culture medium contains serum, each flask should be rinsed with D-PBS twice prior to adding trypsin or trypsin-EDTA.
4. Using 1 mL for every 25 cm², add the appropriate volume of trypsin or trypsin-EDTA to each vessel (e.g., each T-25 vessel would be dissociated with 1 mL trypsin or trypsin-EDTA).
5. Gently rock each flask to ensure complete coverage of the trypsin or trypsin-EDTA over the cells.
6. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within about 3 to 6 minutes), remove the flask from the microscope and gently tap the culture vessel from several sides to promote detachment of the cells from the flask. Do not over-trypsinize as this will damage the cells.
 - a. Some strongly adherent cell types, such as keratinocytes, may take much longer and may require trypsinization at 37°C.
 - b. Some cell types may require more vigorous tapping.
7. When the majority of cells appear to have detached, quickly add 1 to 2 mL diluted Soybean Trypsin Inhibitor to each vessel for every 25 cm² of surface area. Gently pipette or swirl the culture to ensure all of the trypsin or trypsin-EDTA has been neutralized.
8. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture vessel.

OPTIONAL RINSE STEP:

When needed, add 3 to 5 mL D-PBS to the tissue culture vessel to collect any additional cells that might have been left behind. Transfer the cells and D-PBS to the centrifuge tube containing the dissociated cells. Repeat this rinse step as needed until all cells have been collected.

9. Centrifuge the cells at 125 x g for 5 to 10 minutes.

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10. Aspirate neutralized dissociation solution and resuspend the cell pellet in 2 to 8 mL fresh, pre-warmed, complete growth medium.
 11. Count the cells and seed new culture vessels at the recommended density.
 12. Place newly seeded vessels in a 37°C, 5% CO₂, incubator, and incubate for at least 24 to 48 hours before processing the cells further.
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Quality Control Specifications

Bacterial and fungal testing: Not detected

Mycoplasma contamination: Not detected

Osmolality: 270 to 290 mOsm/kg

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Soybean Trypsin Inhibitor (ATCC 30-2104)

References

References and other information relating to this material are available at www.atcc.org.

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