ATCC

Product Sheet

Soybean Trypsin Inhibitor (ATCC® 30-2104™)

Please read this FIRST

Storage Temp.
When stored at -20°C, the product is stable until the expiration date on the label.

Biosafety Level
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Description

Product 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.
Volume: 20 mL (5 mg/mL)

Directions for Use

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 cm^2, 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

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Or contact your local distributor

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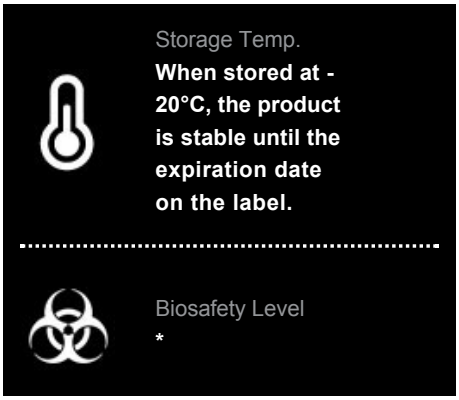

this will damage the cells.

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 for every 25 cm^2 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.
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% CO_2, incubator, and incubate for at least 24 to 48 hours before processing the cells further.

Quality Control Specifications

Lot specific results are available in the Certificate of Analysis, which is available at www.atcc.org or by contacting ATCC technical service.

Test*	Specification
Osmolality	270 to 290 mOsm/kg
Appearance	Clear solution
Sterility testing	Negative for bacteria, fungi, yeast

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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