

ReagentPack™ Subculture Reagents

Introduction

Clonetics™ ReagentPack™ provides the solutions required for successful subculture of primary cells. It contains 100 ml each of HEPES Buffered Saline Solution, Trypsin/EDTA, and Trypsin Neutralizing Solution (TNS). HEPES-BSS is used to neutralize the complex proteins in growth medium that may inactivate trypsin during trypsinization.

Trypsin/EDTA is used to remove cells from culture vessel surfaces. TNS is used to effectively neutralize Trypsin/EDTA.

Stability and Storage

Store at -20°C until labeled expiration. If you plan to use within 3 days, store at 4°C. Trypsin/EDTA has a limited shelf life or activation at 4°C. To keep Trypsin/EDTA fresh and active after thawing, you may aliquot and refreeze at -20°C. Repeated freezing and thawing is not recommended. We recommend that the HEPES-BSS and the Trypsin Neutralizing Solution be stored at 4°C for no more than one month.

Instructions for Use

Based on a 25 cm² flask – adjust volumes accordingly for other size flasks or well plates.

1. Rinse the cells with 5 ml of room temperature Biowhittaker™ HEPES Buffered Saline Solution.
2. Aspirate the HEPES-BSS from the flask.
3. Cover the cells with 2 ml of room temperature Trypsin/EDTA.
4. Examine the cell layer microscopically.
5. Allow the trypsinization to continue until approximately 90% of the cells are rounded up. The entire process takes about 2-6 minutes, depending on the cell type.
6. At this point, rap the flask against the palm of your hand to release the majority of the cells from the culture surface. If only a few cells detach, you may not have let them trypsinize long enough. Wait 30 seconds and rap again. If cells still do not detach, wait and rap every 30 seconds thereafter.

7. After cells are released, IMMEDIATELY neutralize the trypsin in the flask with 4 ml of room temperature Trypsin Neutralizing Solution.

Quality Control

Each lot of HEPES Buffered Saline Solution, Trypsin/EDTA, and Trypsin Neutralizing Solution is standardized for optimal performance and is sterility tested.

Ordering Information

CC-5034	ReagentPack™ Containing: Trypsin/EDTA, 100 ml HEPES Buffered Saline Solution, 100 ml Trypsin Neutralizing Solution, 100 ml
CC-5012	Trypsin/EDTA, 100 ml
CC-5002	Trypsin Neutralizing Solution, 100 ml
CC-5022	HEPES Buffered Saline Solution, 100 ml
CC-5024	HEPES Buffered Saline Solution, 500 ml

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information, or technical service, you may contact Lonza by web, e-mail, telephone, fax or mail.

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