

Production Protocol for Preparing EXO/SAP Mixture

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Version 1.2

PURPOSE: This protocol describes how to make and store the EXO/SAP mixture that is used to clean-up PCR products for sequencing.

MATERIALS AND EQUIPMENT:

EXO I enzyme at 10Units/uL

SAP enzyme at 1Unit/uL

Sigma Water

ddH₂O

Benchtop Minifuge

-20°C Freezer

bucket of ice

50mL Conical tube

Ice

Pipettes and corresponding filter tips of varying sizes

4°C Refrigerator

Aluminum Foil

Tape

Permanent Marker

PROCEDURE:

1. Making the EXO/SAP Mixture

- 1.1. Obtain each of the following: Styrofoam cooler full of ice, pipettes, pipette filter tips, and a new bottle of Sigma Water.
- 1.2. Wrap a new 50mL conical tube with aluminum foil to protect the EXO/SAP mix from light. Label the tube with tape that indicates the contents, batch number, who made the batch, and when the batch was made.
- 1.3. Place the foil-wrapped conical on ice.
- 1.4. 2uL of EXO/SAP mix will be added per well, so for each reaction combine the following reagents in the foil-wrapped conical tube:
 - 1.45uL Sigma Water
 - 0.37uL EXO I @ 10units/uL
 - 0.18uL SAP @ 1unit/uL
 - 2.00uL
- 1.5. First, add the appropriate amount of Sigma Water to the conical tube and place on ice.
- 1.6. Take the bucket of ice along to the freezer and retrieve the appropriate number of tubes of EXO I and SAP.
- 1.7. After recording the lot numbers, spin the tubes in a bench top minifuge to be sure all enzyme is at the bottom of each tube.
- 1.8. Add the appropriate amount of SAP to the conical tube on ice. Immediately return the SAP to the ice.
- 1.9. Add the appropriate amount of EXO I to the conical tube on ice. Mark the stock tubes of EXO I and SAP to indicate to the next user that they have been opened. Immediately return any unused EXO I and SAP to the -20°C freezer.
- 1.10. Cap the conical tube and gently invert it several times.
- 1.11. **The EXO/SAP mix should be passed off and used immediately since the shelf life is only 7 days at 4°C. The EXO/SAP mix should be stored in the back of a 4C refrigerator unless it is being used.**

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Revisions:

6/29/05: Sigma Water was added to the Materials and Equipment Section.

dH2O was removed as an additive to the EXO/SAP brew recipe.

Sigma Water was added to the EXO/SAP brew recipe.

Changed grammar and wording throughout protocol.