

PCR product cleaning with ExoSAP

ExoSAP is a cheap and convenient method for direct PCR product cleaning. 1 U Exonuclease I (New England Biolabs, order number: M0293S; 3000 Units with 20 U/ μ l) and 1 U Shrimp Alkaline Phosphatase (USB Amersham, order number: E70092X; 5000 Units with 1 U/ μ l) are used for 5 μ L of a PCR reaction product. Alternatively you may use the more expensive ExoSAP-IT Kit from USB Amersham (order number: US78200).

- **Exonuclease I:** for 5 μ L PCR reaction product 1U Exonuclease I (*E. coli*) is used. It is recommended to make small aliquots of 1:20 diluted Exonuclease I, because the enzymes activity decreases quite rapidly by thawing and unthawing.

Exonuclease I shipping concentration 20,000 U/ml = 20 U/ μ l

Exonuclease I 1:20 working solution (=1U/ μ l; for 100 μ l working solution):

5 μ l Exonuclease I (shipping solution)

+85 μ l TE-Buffer pH 7.5

+10 μ l Glycerin (ca.87%)

Store this Exonuclease I working solution at -20°C in small aliquots.

- **Shrimp Alkaline Phosphatase:** for 5 μ L PCR reaction product 1U enzyme is used.

- Per cleaning reaction use:
 - 5 μ l PCR product
 - 1 μ l Exonuclease I (working solution)
 - 1 μ l Shrimp Alkaline Phosphatase (shipping solution)total volume: 7 μ l

- Cycling program*:
 - 30 min / 37°C
 - 15 min / 80°C
 - Stop / 4°C

* do not heat the top cover of the thermocycler

- Spin PCR tubes shortly.
- Spa sequencing reaction:
 - 1.0 μ l Premix*
 - 1.5 μ l 5x Sequencing Buffer**
 - 2.0 μ l primer (10 pmol)
 - 2.0 μ l cleaned PCR product
 - 3.5 μ l HPLC water

total volume: 10.0 μ l

*ABI Prism BigDye Terminator 3.x Ready Reaction Cycle Sequencing Kit, **400mM Tris-HCl; 10mM MgCl₂

