**Purification of PCR Products before Sequencing**

Before the performance of the cycle sequencing reaction the PCR products must be purified to remove free primers and dNTPs, which might influence the sequencing reaction.
For the enzymatic purification ExoSAP-IT® is recommended. This enzyme combination ensures that the free primers and dNTPs are digested.

Protocol for the purification with ExoSAP-IT®:

| | |
|---|---|
| 1. | Remove lids of the plates. |
| 2. | Use a multipipette to add 2µl of ExoSAP-IT® (1µl of ExoSAP-IT® for half-volume set-up) to each sample. To ensure that every sample contains ExoSAP-IT® pipette the enzyme just below the upper rim of the well. Check carefully for completeness after pipetting. |
| 3. | Close plate, and spin down briefly to ensure that the enzyme mix is resolved in the sample. |
| 4. | Afterwards mix (vortex) carefully and spin down briefly again. |
| 5. | Incubate samples in a thermocycler with the program given below. |
| 6. | After the incubation step dilute samples 1:1 or up to 1:3 with highly purified water. |
| 7. | Document purification step in the PCR Protocol |

Instead of ExoSAP-IT® a mixture of Alkaline Phosphatase and Exonuclease I can be used, as provided with your sample chemistry.
Please mix 0,4µl of Exonuclease I and 1,6µl of Alkaline Phosphatase per sample and use it as described above for ExoSAP-IT®. Use 1µl of the mixture for half-volume set-up.

Thermocycler incubation program for ExoSAP-IT® / Mixture of Exonuclease I and of Alkaline Phosphatase:

| Incubation: | Enzyme inactivation | |
|---|---|---|
| 1 Cycle:<br>37℃: 15 min. | 1 Cycle:<br>85℃: 15 min. | 10℃ ∞ |