PCR Clean-up Spin Protocol

1. Add a 3× reaction volume of Buffer PCR-A to the sample. If the required volume of Buffer PCR-A is less than 100 µl, add 100 µl of Buffer PCR-A. Vortex briefly to mix the contents.
   Note: It is not necessary to remove mineral oil from the PCRs. Do not include the mineral oil volume in calculating the required volume of Buffer PCR-A.
   Examples: For a 20 µl PCR (with or without oil overlay) add 100 µl of Buffer PCR-A. For a 40 µl PCR, add 120 µl of Buffer PCR-A.

2. Place a PCR column into a 2 ml Microfuge tube (provided). Pipette the reaction from Step1 into the PCR column. Centrifuge at 12,000×g for 1 minute.

3. Discard the filtrate from the 2 ml Microfuge tube. Return the PCR column to the 2 ml Microfuge tube. Pipette 700 µl of Buffer W2 into the column and centrifuge at 12,000×g for 1 minute.

Note: Make sure that the volume of ethanol specified on the bottle label has been added to the Buffer W2 concentrate.

4. Discard the filtrate. Return the PCR column to the 2 ml Microfuge tube. Pipette 400 µl of Buffer W2 into the column and centrifuge at 12,000×g for 1 minute.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

5. Transfer the PCR column into a clean 1.5 ml Microfuge tube (provided). To elute the DNA, add 25-30 µl of Eluent (pre-warmed at 65°C) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000×g for 1 minute.

Note: Deionized water can also be used for elution.