AxyPrep PCR Clean-up Kit

AxyPrep-96 PCR Clean-up Kit

For the purification of amplicons from PCRs

Kit contents, storage and stability

AxyPrep PCR Clean-up Kit

Cat. No.	AP-PCR-4	AP-PCR-50	AP-PCR-250
Kit size	4 preps	50 preps	250 preps
PCR column	4	50	250
1.5 ml Microfuge tube	4	50	250
2 ml Microfuge tube	4	50	250
Buffer PCR-A	2 ml	20 ml	100 ml
Buffer W2 concentrate	2.4 ml	24 ml	2×72 ml
Eluent	1 ml	5 ml	25 ml
Protocol manual	1	1	1

AxyPrep-96 PCR Clean-up Kit

Cat. No.	AP-96-PCR-1	AP-96-PCR-4	AP-96-PCR-24
Kit size	1×96 preps	4×96 preps	24×96 preps
96-well PCR plate	1	4	24
96-well 1.6 ml growblock	1	4	24
96-well V-bottom sample plate	1	4	24
Buffer PCR-A	40 ml	2×80 ml	2×480 ml
Buffer W2 concentrate	72 ml	2×72 ml	6×150 ml
Eluent	10 ml	25 ml	110 ml
Protocol manual	1	1	1

All buffers are stable for at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight and extremes in temperature.

Buffer PCR-A: DNA binding buffer. Store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before the use of the kit, add the volume of ethanol specified on the bottle label and mix well. Either 100% or 95% denatured ethanol can be used. Store at room temperature.

Eluent: 2.5 mM Tris-HCl, pH 8.5. Store at room temperature.

Introduction

The AxyPrep PCR Cleanup kits employ a special Binding Solution in combination with an Axyprep PCR column and 96-well plate having an optimized chromatographic membrane to achieve high selectivity and recovery of linear DNA fragments. This product is designed to purify DNA fragments longer than 75 bp from PCRs and other enzymatic reactions, with an expected recovery of 70-90%. Each column/well has a binding capacity of up to 8 μg. It is not necessary to remove mineral oil overlays from the PCRs before purification. This protocol will remove unincorporated primers (<50 nt), enzymes and unlabeled mononucleotides. The purified DNA fragments are suitable for a variety of applications, such as sequencing, ligation, restriction analysis, *in vitro* transcription, microinjection and microarrays.

Caution

Buffer PCR-A contains a chemical irritant. When working with the buffer, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and supplies required but not provided

- Microcentrifuge capable of 12,000×g
- Table top centrifuge with swinging bucket rotor and plate carriers (for 96 preparation only)
- Vacuum manifold with luer-type fittings (#AP-VM)
- Vacuum source and regulator (-25-30 inches Hg required)
- Heated water bath
- 95-100% ethanol

Preparation before experiment

1) Before using the kit, add the specified volume of ethanol to the Buffer W2 concentrate. Either 100% or 95% (denatured) ethanol may be used.
2) Check Buffer PCR-A for precipitation before each use. If precipitation occurs, warm at 65°C to dissolve the precipitate.
3) Pre-warming Eluent to 65°C will generally improve elution efficiency.

Protocols

I. AxyPrep PCR column

PCR Clean-up Vacuum Protocol

Any vacuum manifold with complementary fittings, such as the AxyVac Vacuum Manifold can be used with the PCR columns. A negative pressure of –20-25 inches Hg is required. We recommend the use of a vacuum regulator to adjust the negative pressure. -25-30 inches Hg is equivalent to approximately -850-1,000 mbar and -12-15 psi.

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. Attach the vacuum manifold to the vacuum source. Insert a PCR column into one of the complimentary fittings. Pipette the reaction from Step1 to a PCR column. Switch on vacuum source and adjust to –25-30 inches Hg. Continue to apply vacuum until no liquid remains in the PCR column.
3. Add 700 μl of Buffer W2 along the wall of the PCR column to wash off residual salt. Initiate vacuum and apply until no liquid remains in the column. Repeat this wash with a second 700 μl aliquot of Buffer W2.
   Note: Make sure that the volume of ethanol specified on the bottle label has been added to the Buffer W2 concentrate.
4. Transfer the PCR column into a 2 ml Microfuge tube (provided) and centrifuge at 12,000×g for 1 minute.
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.

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.

II. AxyPrep 96-well plate

96 PCR Clean-up Vacuum Protocol

Any vacuum manifold which can accommodate 96-well plates, such as the AxyVac Vacuum Manifold (AP-VM) can be used with the AxyPrep 96-well PCR plate. A negative pressure of –25-30 inches Hg is required. We recommend the use of a vacuum regulator to adjust the negative pressure. -25-30 inches Hg is equivalent to approximately -850-1,000 mbar and -12-15 psi.

1. Add a 3× reaction volume of Buffer PCR-A to each sample. If the required volume of Buffer PCR-A is less than 100 μl, add 100 μl of Buffer PCR-A. Vortex briefly or pipette up and down 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. Attach the vacuum manifold to vacuum source. Place an AxyPrep 96-well PCR plate onto the vacuum manifold. Transfer the diluted reactions from Step 1 to the corresponding wells of the 96-well PCR plate. Switch on vacuum source and adjust to –25-30 inches Hg. Continue to apply vacuum until no liquid remains in any of the plate wells.
3. Pipette 300 μl of Buffer W2 along the wall of each well to remove residual salt solution. Continue to apply vacuum until no liquid remains in any of the plate wells. Repeat this wash step TWICE with 300 μl of Buffer W2.
   Note: Make sure that the volume of ethanol specified on the bottle label has been added to the Buffer W2 concentrate.

4. Continue to draw the vacuum through the PCR plate for 10 minutes to dry the membrane.

5. Turn the vacuum source off and release the vacuum inside. Remove the PCR plate. Hold the plate in the normal upright position and tap 6× on absorbent toweling vigorously to remove any residual Buffer W2 from the underside of the plate.

   Note: Be careful not to damage the drip directors when tapping.

   Note: When purifying DNA fragments intended for automated fluorescent (capillary) sequencing, we recommend the use of "lint-free" absorbent toweling.

6. Transfer the AxyPrep PCR plate onto a 96-well V-bottom sample plate (provided). Add 25-30 μl of Eluent, pre-warmed at 65°C to the center of membrane, and let it stand at room temperature for 1 minute. Centrifuge at ⩾3,000×g for 5 minutes to elute the DNA.

   Note: Pre-warming Eluent at 65°C will often improve elution efficiency.

   Note: Deionized water can be used for elution.

96 PCR Clean-up Spin Protocol

This procedure requires the use of a low-speed centrifuge with a swinging bucket rotor and plate carriers.

1. Add a 3× reaction volume of Buffer PCR-A to each sample. If the required volume of Buffer PCR-A is less than 100 μl, add 100 μl of Buffer PCR-A. Vortex briefly or pipette up and down 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 AxyPrep PCR plate onto a 1.6 ml growblock (provided). Transfer the diluted reactions from Step 1 to the corresponding wells of the PCR plate. Place the PCR plate and 1.6 ml growblock into the plate carrier(s) of a swinging bucket rotor. Centrifuge at 1,000×g for 1 minute.

   Note: Following this centrifugation step, it is not necessary to discard the filtrate from the 1.6 ml growblock.

3. Remove the PCR plate from the centrifuge. Add 300 μl of Buffer W2 along each well of the PCR plate. Reposition the PCR plate on the 1.6 ml growblock and centrifuge at 1,000×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. Repeat this wash step with a second 300 μl aliquot of Buffer W2.
5. Discard the filtrate from the 1.6 ml growblock. Place the PCR plate back onto the 1.6 ml growblock. Centrifuge at ⩾3,000×g for 10 minutes to removal residual wash solution from the plate.

   Note: Only prolonged centrifugation at >3,000×g can ensure the complete removal of Buffer W2 from the PCR plate membranes.

6. Transfer the AxyPrep PCR plate onto a V-bottom sample plate (provided). Add 25-30 μl of Eluent (pre-warmed at 65°C) to the center of membrane, and let it stand at room temperature for 1 minute. Centrifuge at ⩾3,000×g for 5 minutes to elute the DNA.

   Note: Pre-warming Eluent at 65°C will often improve elution efficiency.

   Note: Deionized water can be used for elution.

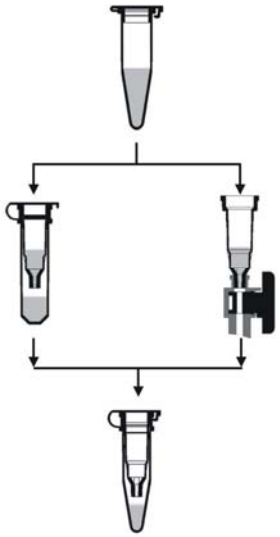
Overviews

I. AxyPrep PCR column

Add 3× sample volume of Buffer PCR-A.
If the required volume is less than 100 μl, add 100 μl of Buffer PCR-A

Add 700 μl of Buffer W2
Repeat wash with Buffer W2

Add 25-30 μl of Eluent or deionized water



Binding

Washing

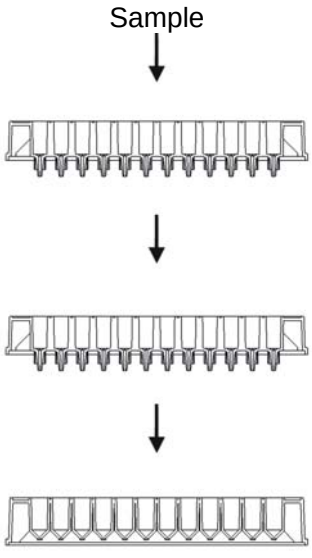
Elution

II. 96-well plate

Add 3× sample volume of Buffer PCR-A.
If the required volume is less than 100 μl, add 100 μl of Buffer PCR-A

Add 300 μl of Buffer W2
Repeat wash with Buffer W2

Add 25-30 μl of Eluent or deionized water



Binding

Washing

Elution

Troubleshooting

1. Low or no recovery

Ethanol not added to Buffer W2 concentrate

Either 100% or 95% denatured ethanol must be added to Buffer W2 concentrate before use. Repeat procedure with correctly prepared Buffer W2.

Inappropriate elution buffer

DNA will only be eluted efficiently in the presence of low salt and pH 7.0. Use the provided eluent (2.5 mMTris-HCl, pH 8.5) or water for elution. Warm eluent to 65°C to improve elution efficiency.

Elution buffer incorrectly dispensed

Dispense the elution buffer onto the center of each well to ensure that the membrane is evenly saturated.

Insufficient vacuum

Symptoms of insufficient vacuum include reduced eluate volume (<20 μl recovered from 30 μl) or eluate volume varying between wells. Make sure vacuum source is generating –25-30 inches Hg.

2. DNA does not perform well in downstream applications

Salt concentration in eluate too high

Ensure that the wash steps are carried out before elution to remove salt.

Eluate contains residual ethanol (samples float out of wells of agarose gel)

Carefully follow all steps in the protocol to remove residual ethanol.

Eluate contains primer-dimers

Primer dimers may interfere with subsequent reactions, such as sequencing. Any primer-dimers >40 bp will not be completely removed. After the binding step, wash the AxyPrep PCR column or plate with 750 μl of 35% guanidine hydrochloride (35 g in 100 ml deionized water). Continue with the Buffer W2 wash and the elution step as described in the protocol.

Eluate contains denatured single-stranded DNA which appears as a smaller smeared band on an analytical gel

After elution, heat the sample at 95°C for 2 minutes. Allow to cool slowly to room temperature before use. The sample can also be supplemented with 10 mM NaCl to promote renaturation after heating 95°C for 2 minutes.