AGENCOURT® AMPURE® XP

PCR PURIFICATION

Please refer to http://www.agencourt.com/technical for updated protocols and refer to MSDS instructions http://www.beckmancoulter.com/customersupport/msds/msds.asp when handling or shipping any chemical hazards.

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Introduction

The Agencourt AMPure XP PCR[1] Purification systems utilize Agencourt's solid-phase paramagnetic bead technology for high-throughput purification of PCR amplicons. Agencourt AMPure XP utilizes an optimized buffer to selectively bind PCR amplicons 100bp and larger to paramagnetic beads. Excess primers, nucleotides, salts, and enzymes can be removed using a simple washing procedure. The resulting purified PCR product is essentially free of contaminants.

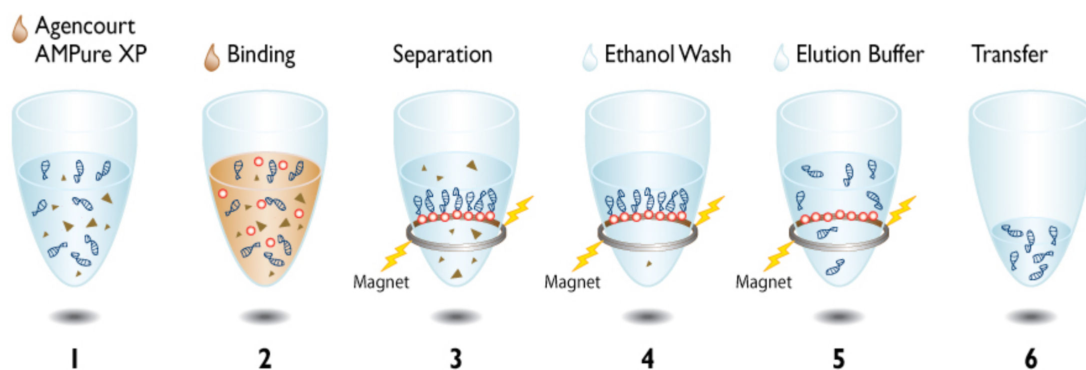
[1] *The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*

Agencourt AMPure XP purified products can be used in the following applications:

- PCR
- Sequencing (Sanger and Next Generation)
- Genotyping and SNP detection
- Fragment Analysis
- Primer Walking
- Cloning

The purification procedure is highly amenable to a variety of automation platforms because it utilizes magnetic separation and requires no centrifugation or vacuum filtration. More information on automating Agencourt XP can be found at http://www.agencourt.com/technical/reagent_information//

Process Overview



1. Add 1.8ul AMPure XP per 1.0ul of PCR product
2. Bind PCR products to paramagnetic beads
3. Separation of beads + PCR from contaminants
4. Wash beads + PCR product 2x with 70% Ethanol to remove contaminants
5. Elute purified PCR product from beads
6. Transfer to new plate

For questions regarding this protocol, please contact Technical Support at Agencourt at support@agencourt.com or 1-800-773-9186

Kit Specifications

The Agencourt AMPure XP PCR purification kit can be used in 96 and 384 well format. The following table illustrates the number of PCR reactions an Agencourt AMPure XP kit will purify depending on the format required by the user.

AMPure XP

AMPure XP Product	Product #
AMPure XP 5.0mL	A63880
AMPure XP 60 mL	A63881
AMPure XP 450 mL	A63882

PCR Reaction Volume 96 Well Format (µL)	Product # A63880	Product # A63881	Product # A63882
10	278 rxns	3332 rxns	25000 rxns
20	139 rxns	1666 rxns	12500 rxns
50	56 rxns	667 rxns	5000 rxns
100	28 rxns	334 rxns	2500 rxns

PCR Reaction Volume 384 Well Format (µL)	Product # A63880	Product # A63881	Product # A63882
5	556 rxns	6667 rxns	50000 rxns
7	397 rxns	4762 rxns	35714 rxns
10	278 rxns	3333 rxns	20000 rxns
14	198 rxns	2381 rxns	17857 rxns

Materials Supplied in the Kit:

Agencourt AMPure XP Magnetic Particle Solution

- Store at 4°C upon arrival, for up to 12 months
- Mix the reagent well before use. It should appear homogenous and consistent in color
- DO NOT FREEZE

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Materials Supplied by the User:

Consumables and Hardware:

- Reaction Plate:
  For 96 well format: 96 well (300 µL well capacity) round bottom plate [For 96 well format: 96 well 300 µL round bottom microtiter plate [Costar* # 07-200-105; www.fishersci.com] or 96 well cycling plate [ABgene* product # AB-0800; AB-1000 or AB-1400 http://www.abgene.com/]

  For 384 well format: 384 well (40 µL well capacity) cycling plate [For Automation: Hard-Shell* PCR plate # HSP-3801; 07-200-105]; [ABgene product # AB-1111 http://www.abgene.com/]

- Agencourt SPRIPlate® magnetic plate:
  For 96 well format: Agencourt SPRIPlate 96 Ring Super Magnet Plate [Agencourt product # A32782; http://www.agencourt.com/]
  For 384 well format: Agencourt SPRIPlate 384 [Agencourt product # A29165; http://www.agencourt.com/]
- Plate Seals, adhesive or heat. [for example: ABgene product # AB-3739; http://www.abgene.com/]
- Liquid handling robotics or a multichannel hand pipette

Reagents:

- Fresh 70% ethanol (***Note: 70% ethanol is hygroscopic. Fresh 70% ethanol should be prepared for optimal results***)
- 10 mM TRIS-Acetate, pH 8.0, reagent grade water or, TE Buffer [10 mM Tris-Acetate pH 8.0, 1 mM EDTA] for DNA elution

Calculation of Percent Recovery:

To gauge percent recovery, analyses of the samples pre-purification and post-purification are necessary. For this process, Agencourt recommends either a PicoGreen®[2] assay or visualization on agarose gel. Spectrophotometric analysis using Optical Density (OD) at 260 nm is discouraged because at 260 nm both single and double-stranded nucleic acids will contribute to the overall absorbance reading. For the pre-purification sample, single-stranded PCR primers and dNTPs will contribute to the initial absorbance and give a falsely inflated reading of the quantity of PCR product. By contrast, the PicoGreen assay uses an intercalating dye to specifically quantitates only double-stranded DNA. When taking a PicoGreen reading pre-purification, PCR

[2] *PicoGreen is available from Molecular Probes® http://www.probes.com/*

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primers and dNTPs will not falsely inflate the reading. This enables a more accurate quantification of recovery. In addition to PicoGreen readings, visualization of the sample pre- and post-purification on agarose gel with ethidium bromide is recommended, but would be more subjective. For most accurate results, run both pre- and post-purified samples on the same gel to minimize differences in electrophoresis parameters and imaging processes.

Procedure:

96 Well Format:

1. **Determine whether or not a plate transfer is necessary.**

   If the PCR reaction volume multiplied by 2.8 exceeds the volume of the PCR plate, a transfer to a 300 µL round bottom plate is required..

2. **Gently shake the Agencourt AMPure XP bottle to resuspend any magnetic particles that may have settled. Add Agencourt AMPure XP according to the PCR reaction volume chart below:**

PCR Reaction Volume (µL)	AMPure XP Volume (µL)
10	18
20	36
50	90
100	180

   The volume of Agencourt AMPure XP for a given reaction can be derived from the following equation: (Volume of Agencourt AMPure XP per reaction) = 1.8 x (Reaction Volume)

3. **Mix reagent and PCR reaction thoroughly by pipette mixing 10 times. Let the mixed samples incubate for 5 minutes at room temperature for maximum recovery.**

   This step binds PCR products 100bp and larger to the magnetic beads. Pipette mixing is preferable as it tends to be more reproducible. The color of the mixture should appear homogenous after mixing.

4. **Place the reaction plate onto an Agencourt SPRIPlate 96 Super Magnet Plate for 2 minutes to separate beads from the solution.**

   Wait for the solution to clear before proceeding to the next step.

5. **Aspirate the cleared solution from the reaction plate and discard.**

   This step must be performed while the reaction plate is situated on the Agencourt SPRIPlate 96 Super Magnet Plate. Do not disturb the ring of separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

6. **Dispense 200 µL of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate out the ethanol and discard. Repeat for a total of two washes.**

   It is important to perform these steps with the reaction plate situated on an Agencourt SPRIPlate 96 Super Magnet Plate. Do not disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well as it is a known PCR inhibitor.

   NOTE: *A dry time of ≤5 min at Room Temperature is optional to ensure all traces of Ethanol are removed but take care not to over dry the bead ring (bead ring appears cracked) as this will significantly decrease elution efficiency.*

7. **Off the magnet plate, add 40 µL of elution buffer (Reagent grade water, TRIS-Acetate pH 8.0, or TE) to each well of the reaction plate and pipette mix 10 times.**

   The liquid level will be high enough to contact the magnetic beads at a 40 µL elution volume. A greater volume of elution buffer can be used, but using less than 40 µL will require extra mixing (to ensure the liquid comes into contact with the beads) and may not be sufficient to elute the entire PCR product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

8. **Place the reaction plate onto an Agencourt SPRIPlate 96 Super Magnet Plate for 1 minute to separate beads from the solution.**

9. **Transfer the eluant to a new plate.**

   For long term freezer storage, Agencourt recommends transferring Agencourt AMPure XP purified samples into a new plate to prevent beads from shattering.

For questions regarding this protocol, please contact Technical Support at Agencourt at support@agencourt.com or 1-800-773-9186

384 Well Format:

1. **Shake the Agencourt AMPure XP bottle to resuspend any magnetic particles that may have settled. Add Agencourt AMPure XP according to the following PCR reaction volume chart:**

PCR Reaction Volume (µL)	Agencourt AMPure XP or Agencourt AMPure XP XL Volume (µL)
5	9
7	12.6
10	18
14	25

The volume of Agencourt AMPure XP for a given reaction can be derived from the following equation: (Volume of Agencourt AMPure XP per reaction) = 1.8 x (Reaction Volume)

NOTE: *Due to the total volume of PCR reaction plus reagent, it is not possible to purify PCR reactions larger than 14 µL within the well of 384 well plates (14 µL reaction + 25 µL Agencourt AMPure XP = 39 µL).*

2. **Mix reagent and PCR reaction thoroughly.**

Pipette mix 15 times. The color of the mixture should appear homogenous after mixing.

3. **Place the reaction plate onto an Agencourt SPRIPlate 384 for 3 minutes to separate the beads from solution.**

The solution should be clear before proceeding to the next step.

4. **Aspirate the cleared supernatant from the reaction plate and discard.**

This step should be performed while the purification plate is situated on the Agencourt SPRIPlate 384. Do not touch the magnetic beads, which have formed a spot on the side of the well.

5. **Dispense 30 µL of 70% ethanol wash solution to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate the ethanol out and discard. Repeat for a total of two washes.**

It is important to perform these steps with the reaction plate situated on an Agencourt SPRIPlate 384. Do not disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well as it is a known PCR inhibitor.

NOTE: *A dry time of ≤5 min at Room Temperature is optional to ensure all traces of Ethanol are removed but take care not to over dry the bead ring (bead ring appears cracked) as this will significantly decrease elution efficiency.*

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6. **Off the magnet plate, add 30 µL of elution buffer (Reagent grade water, TRIS-Acetate pH 8.0, or TE) to each well and pipette mix 10 times.**

A 30 µL elution volume will ensure the liquid level will be high enough to contact the magnetic beads. A greater volume of elution buffer can be used, but using less than 15 µL requires extra mixing (to ensure the liquid comes into contact with the beads) and may not fully elute the entire product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

When setting up downstream reactions, pipette the DNA from the plate while it is situated on the Agencourt SPRIPlate 384. This will prevent bead carry over (however, beads will not inhibit thermal cycling reactions). For long term freezer storage, Agencourt recommends transferring Agencourt AMPure XP purified samples into a new plate to prevent beads from shattering.

For information on automating the Agencourt AMPure XP process, please visit ***http://www.agencourt.com/technical***

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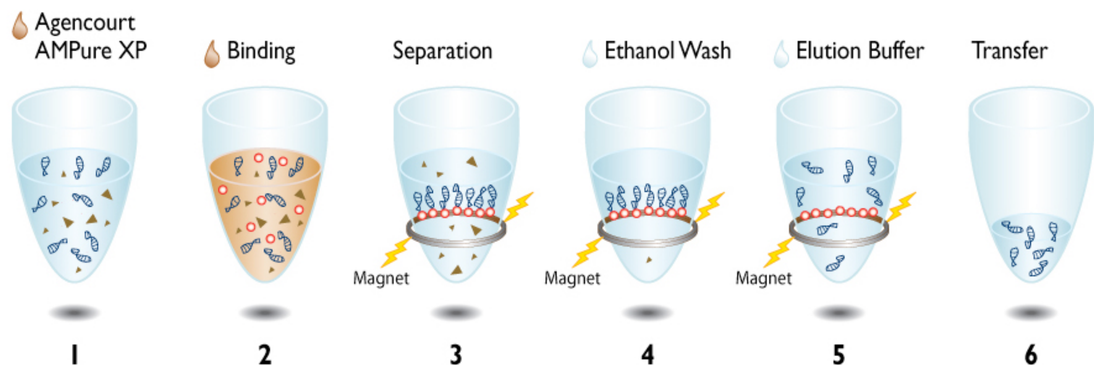
Agencourt AMPure XP Quick Reference for 96 Well Format

1. **Sample volume X 2.8 > Well volume? □ Yes, goto step 2 □ No, goto step 3**
2. **Transfer sample to a 300 µL round bottom plate.**
3. **Shake the Agencourt AMPure XP bottle to fully resuspend magnetic particles.**
4. **Add Sample Vol µL X 1.8 of Agencourt AMPure XP. Pipette mix 10 times.**
5. **Incubate at room temperature for 5 minutes.**
6. **Place the reaction plate onto an Agencourt SPRIPlate Super Magnet Plate for 2 minutes to separate beads from solution.**
7. **Aspirate the supernatant from the reaction plate and discard.**
8. **Dispense 200 µL of 70% ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. Repeat for a total of two washes.**
9. **Add ≥40 µL of elution buffer, pipette mix 10 times.**
10. **Incubate at room temperature for 2 minutes.**
11. **Place the reaction plate onto an Agencourt SPRIPlate Super Magnet Plate for 1 minute to separate beads from solution.**
12. **Transfer purified product to a new plate**



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