AGENCOURT® AMPURE®

PCR PURIFICATION

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

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

- Fluorescent DNA sequencing, including capillary electrophoresis
- Microarray spotting[2]
- Cloning[2]
- Primer extension genotyping

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

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

[2] Please e-mail or call Agencourt support for beta protocols (support@agencourt.com; 1-800-773-9186)

information on automating the Agencourt AMPure can be found at http://www.agencourt.com/technical/reagent_information//.

Process Overview

The Agencourt AMPure procedure is performed in three stages:

- Selective binding of PCR products to paramagnetic beads and separation of the beads with a magnetic field
- Washing the beads to remove contaminants
- Eluting the purified PCR products from the magnetic beads

Kit Specifications

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

PCR Reaction Volume 96 Well Format	Product # 000130	Product # 000132
10	3250	25000
20	1625	12500
50	650	5000
100	325	2500
PCR Reaction Volume 384 Well Format	**Product # 000130**	**Product # 000132**
5	6500	50000
7	4642	35714
10	3250	20000
14	2321	17857

Materials Supplied in the Kit:

Agencourt AMPure Magnetic Particle Solution

- Store at 4°C upon arrival, for up to 6 months. Allow Agencourt AMPure to come to room temperature prior to use.
- Mix the reagent well before use. It should appear homogenous and consistent in color.
- DO NOT FREEZE.

Materials Supplied by the User:

Consumables and Hardware:

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

  For 384 well format: 384 well (40µL well capacity) cycling plate [For Automation: MJ Research Hard-Shell™ PCR plate # HSP-3801; http://www.mjr.com/html/consumables/microplates/hard_shell.html]; [ABgene product # AB-0937 (http://www.abgene.com/), will require manual intervention]

- Agencourt SPRIPlate® magnetic plate:
  For 96 well format: Agencourt SPRIPlate magnetic plate: Agencourt SPRIPlate96R ring magnetic plate [Agencourt product # 000219; http://www.agencourt.com/]
  For 384 well format: Agencourt SPRIPlate 384 magnetic plate [Agencourt product # 000222]
- 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 [10mM Tris-Acetate pH 8.0, 1mM 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®[3] assay or visualization on agarose gel. Spectrophotometric analysis using Optical Density (OD) at 260 nm is discouraged. At 260 nm both single and double-stranded nucleic acids will contribute to the

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

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 quantitate only double-stranded DNA. When taking a PicoGreen reading pre-purification, PCR primers and dNTPs will not falsely inflate the reading. This enables a more accurate quantitation of recovery. In addition to PicoGreen readings visualization of the sample pre- and post-purification on agarose gel with ethidium bromide is recommended, but will give a more subjective quantitation. 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 * 2.8 exceeds the volume of the PCR plate, a transfer to a 300 µL round bottom plate is required. Agencourt recommends the Costar 3795 plate to work with the Agencourt AMPure kit, because the Agencourt SPRIPlate96R was designed specifically for the Costar plate. The PCR Reactions can be set up in polypropylene PCR/ thermal cycling plates. The cleanup reaction can be performed in the same plate, if the volume of the PCR reaction is below 71 µL. A 300 µl plate will hold up to 105 µL of sample and 189 µL of Agencourt AMPure.

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

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

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

For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186

3. **Mix the Agencourt AMPure and PCR reaction thoroughly by pipette mixing 10 times or vortexing for 30 seconds.**

   This step binds PCR products 100bp and larger to the magnetic beads. Pipette mixing is preferable as it tends to be more reproducible. If vortexing is used, it is recommended to seal the plate with a plate seal before vortexing. The color of the mixture should appear homogenous after mixing. Let the mixed samples incubate for 3 -5 minutes at room temperature for maximum recovery (optional). This is strongly recommended for reactions 50 µl and larger.

4. **Place the reaction plate onto an Agencourt SPRIPlate 96R for 5 - 10 minutes to separate beads from solution.**

   The separation time is dependent on the size of the reaction. 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 96R. Do not disturb the ring of separated magnetic beads.

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 96R. Do not disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well as it may contain residual contaminants. The ethanol can also be discarded by inverting the plate to decant, but this must be done while the plate is situated on the Agencourt SPRIPlate 96R.

7. **Place the reaction plate on bench top to air-dry. Be sure to allow the plate to dry completely.**

   The plate should be left to air-dry for 10-20 minutes on a bench top to allow complete evaporation of residual ethanol. Longer drying times may be required for microarraying. Alternatively the plate can be incubated at 37°C for faster evaporation. If the samples will be used immediately, proceed to Step 8 for elution. If the samples will not be used immediately, the dried plate may be sealed and stored at 4°C or -20°C.

8. **Add 40 µL of elution buffer (TRIS-Acetate, DiH_2O, or TE) to each well of the reaction plate and seal to vortex 30 seconds or 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 vortexing (to ensure the liquid comes into contact with the beads) and may not be sufficient to fully elute all of the product. 10 mM Tris-Acetate pH 8.0 (recommended), reagent grade water, or TE buffer may be used for the elution. Recommended elution conditions are sealing and vortexing for 30 seconds or 10 pipette mixes. Elution is quite rapid and it is not necessary for the beads to go back into solution for complete elution to occur.

When setting up downstream reactions, pipette the DNA from the plate while it is situated on the Agencourt SPRIPlate96R. This will prevent bead carry over (however, the beads do not inhibit thermal cycling reactions). For long term freezer storage, Agencourt recommends transferring Agencourt AMPure purified samples into a new plate away from the magnetic particles.

384 Well Format:

1. **Transfer the PCR reactions into a 384 well skirted PCR plate.**

   For automation, Agencourt strongly recommends the MJ Research Hard-Shell PCR plate (HSP-3801). The design of this plate virtually eliminates warping caused by thermal cycling, making it easier for robotic systems to move the plates on and off of the Agencourt SPRIPlate384 magnet. Other 384 well plates are compatible with the magnet (for example Marsh AB-0937), but will require manual intervention to move the plates on and off the Agencourt SPRIPlate384.

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

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

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

Note: Due to the total volume of PCR reaction plus Agencourt AMPure, 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 = 39 µL).

3. **Mix the Agencourt AMPure and PCR reaction thoroughly.**

   For maximum binding and recovery the plate must be removed from the magnet plate.* Pipette mix 15 times. The color of the mixture should appear homogenous after mixing. This step binds PCR products 100 bp and larger to the magnetic beads.

   *If your automation platform makes it difficult to have both on-magnet and off-magnet steps, please see the Agencourt AMPure automation guidelines for additional suggestions. http://www.agencourt.com/technical/reagent_information//.

4. **Place the reaction plate onto an Agencourt SPRIPlate384 for 3 -5 minutes to separate the beads from solution.**

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

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

   This step should be performed while the purification plate is situated on the Agencourt SPRIPlate384. Do not touch the magnetic beads, which have formed a spot on the side of the well.

6. **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 SPRIPlate384. Do not disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well as it may contain residual contaminants.

7. **Place the reaction plate on the bench top to air-dry. Be sure to allow the plate to dry completely.**

   The plate should be left to air-dry for 10-20 minutes on a bench top to allow residual ethanol to evaporate completely. Alternatively, the plate can be incubated at 37°C for faster evaporation. If the samples will not be used immediately, the dried plate should be sealed and stored at 4°C or -20°C.

8. **Add 30 µL of elution buffer (TRIS-Acetate, DiH$_2$O, or TE) to each well of the reaction plate and shake.**

   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 vortexing (to ensure the liquid comes into contact with the beads) and may not fully elute the entire product. 10 mM Tris-Acetate pH 8.0 (recommended), reagent grade water, or TE buffer may be used for the elution. The recommended elution conditions are 10

pipette mixes or vortexing the sealed plate for 30 seconds. Elution is rapid and it is not necessary for the beads to go back into solution for complete elution.

When setting up downstream reactions, pipette the DNA from the plate while it is situated on the Agencourt SPRIPlate384. This will prevent bead carry over (however, beads will not inhibit thermal cycling reactions). For long term freezer storage, Agencourt recommends transferring Agencourt AMPure purified samples into a new plate to prevent beads from shattering.

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

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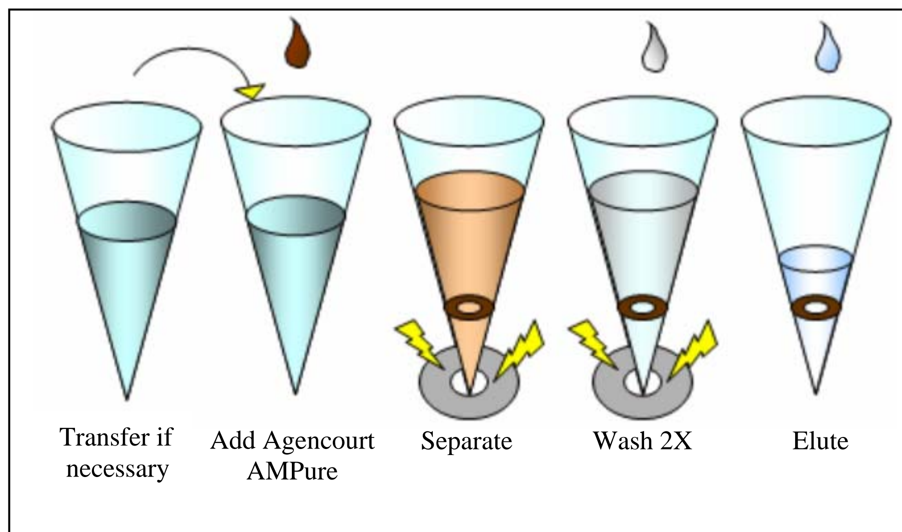
Agencourt AMPure Quick Reference for 96 Well Format:

1. **Plate transfer necessary? □ Yes □ No**
2. **Gently shake the Agencourt AMPure bottle to resuspend any magnetic particles that may have settled.**
3. **Add the correct volume (______ µL) of Agencourt AMPure to the samples. Pipette mix 10 times or vortex for 10 seconds.**
4. **Incubate for ______ minutes.**
5. **Place the reaction plate onto a Agencourt SPRIPlate 96R for ______ minutes to separate beads from solution.**
6. **Aspirate the cleared solution (supernatant) from the reaction plate and discard.**
7. **Dispense 200µL 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.**
8. **Let the reaction plate air-dry for ______ minutes at room temperature.**
9. **Add ______ µL of elution buffer, pipette mix 10 times or vortex for 30 seconds.**



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