

AGENCOURT® AMPURE®

PCR PURIFICATION FOR LARGE VOLUME REACTIONS

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

AGENCOURT AMPURE is a registered trademark of Agencourt Bioscience and is for laboratory use only.

Agencourt AMPure PCR Purification Table of Contents

Agencourt AMPure PCR Purification Table of Contents.....	1
Introduction.....	1
Process Overview	2
Kit Specifications.....	2
Materials Supplied in the Kit:.....	2
Materials Supplied by the User:.....	3
Calculation of Percent Recovery:	3
Procedure:	4
96 Well Format:	4
96 Well Format For Large Volume Reactions (101 – 201 µL):	6
Single Tube Format For Large Volume Reactions (101 – 201 µL):	8
384 Well Format:	10

Introduction

The Agencourt AMPure PCR¹ 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 spotting²
- Cloning²

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)



Innovate Automate
SIMPLIFY

- 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 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.

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

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 PicoGreen³ 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 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

³ PicoGreen is available from Molecular Probes® <http://www.probes.com/>

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

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 for large volume reactions (<101µL):

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)

3. Mix the Agencourt AMPure and PCR reaction thoroughly by pipette mixing 10 times or vortexing for 30 seconds. Allow the plate to incubate for 20 minutes at room temperature. The plate should not be on the magnet at this point.

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

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. Incubation time is required for large volume reactions to allow all the DNA time to bind to the beads. For reactions 100ul and larger, 20 minutes of incubation is required. For reactions 50-100ul, 10 minutes of incubation should be sufficient.

4. Place the reaction plate onto an Agencourt SPRIPlate 96R for 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, DiH₂O, 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

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

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.

96 Well Format For Large Volume Reactions (101 – 201 µL):

Considering the 300 µl capacity of standard 96-well microtiter plates, the following protocol employs two serial AMPure binding reactions/ separation events per sample. Both purifications occur within the same well.

- 1. Transfer half the volume of the unpurified reaction into a 300 µL round bottom microtiter plate.**

Agencourt recommends the Costar 3795 plate to work with the AMPure kit, as the SPRIPlate96R was designed specifically for this plate.

- 2. Based on the half-volume of the reaction, add and mix AMPure according to the chart below:**

<i>Reaction Volume (µL)</i>	<i>AMPure Volume (µL)</i>
25	45
50	90
75	135
100	180

The volume of AMPure for a given reaction can be derived from the following equation:

$$1.8 \times (\text{Reaction Volume}) = (\text{Volume of AMPure per reaction})$$

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

- 3. Mix the AMPure and PCR product thoroughly. Let the tube incubate at room temperature for 5 minutes before proceeding to the next step.**

This step binds the DNA to the magnetic beads. Mix by pipette pipette mixing. Agencourt recommends at least 10 pipette mixes. The color of the mixture should appear homogenous after mixing.

- 4. Place the Purification Plate onto a SPRIPlate96R to separate the beads from solution.**

The recommended separation time is 10 minutes. The solution should be clear before proceeding to the next step. Separation times depend on reaction size volumes; larger PCR reactions will require a longer separation time.

- 5. Aspirate the cleared solution from the plate and discard it.**

This step should be performed with the plate situated on a SPRIPlate96R. Care should be taken not to disturb the ring of separated magnetic beads.

- 6. Dispense the second half of the unpurified reaction into the same well as the first half of the reaction.**

Be careful to get the correct samples in the matching wells. It is no cause for concern, if the beads are disturbed by the addition of the second half of the reaction.

- 7. MAKE SURE THE MICROTITER PLATE IS TAKEN OFF THE MAGNET. Add the appropriate volume of AMPure according to the chart in Step 2 {1.8 µl x (Volume of Second Half of Reaction)}.**

- 8. Mix AMPure and the DNA thoroughly. Let the tube incubate at room temperature for 5 minutes before proceeding to the next step.**

This step binds the DNA to the magnetic beads. Mix by pipette pipette mixing. Agencourt recommends at least 10 pipette mixes. The color of the mixture should appear homogenous after mixing.

- 9. Place the Purification Plate back onto the SPRIPlate96R to separate the beads from solution.**

The recommended separation time is 10 minutes. The solution should be clear before proceeding to the next step. Separation times depend on reaction size volumes; larger PCR reactions will require a longer separation time.

- 10. Aspirate cleared solution from the Purification Plate and discard.**

This step should be performed with the purification plate situated on a SPRIPlate96R. Care should be taken not to disturb the ring of separated magnetic beads.

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

11. Dispense 200 µL of 70% ethanol to each well of the plate. Aspirate the wash solution and discard it . Repeat. Blot the remaining ethanol on paper towels.

It is important to perform these steps while the plate is situated on a SPRIPlate96R. The wash solution should be applied for at least 30 seconds at each step before removal. Care should be taken not to disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well or it may take a long time to dry. You can invert the **plate with the magnet** on a paper towel to remove the remaining ethanol.

12. Leave the 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 15-25 minutes on a bench top to allow the residual ethanol solution to evaporate completely. Alternatively the plate can be incubated at 37°C to allow faster evaporation. After the drying is complete, the plate may be stored dry at 4°C or -20°C. A longer drying time may be required for microarraying. Elute just prior to use.

13. Add 20 µL (or more) of elution buffer to each well of the plate.

Shake (seal the plate prior to shaking) 30 seconds or pipette mix 10 times to resuspend the samples. Let the beads incubate in elution buffer (away from the magnet) for 5 minutes to ensure complete elution.

A linear-motion shaker was used for preliminary testing. Elution volumes can be adjusted, but must be at least 20 µL to effectively elute the DNA from the beads. For elution volumes less than 40 µl, it is recommended to centrifuge the plate briefly in order to concentrate all the liquid to the bottom following the plate shake. 10 mM Tris-Acetate pH = 8.0 (recommended), reagent grade water, or TE buffer may be used for elution.

14. Transfer the sample away from the beads.

To cleanly transfer the samples away from the beads, place the microtiter plate back on the magnet for 15 minutes. Carefully aspirate the cleared sample and dispense it into a clean plate. The 15 minute separation time allows for maximum recovery of sample volume while reducing the risk of bead carry-over.

Single Tube Format For Large Volume Reactions (101 – 201 µL):

1. Transfer PCR reactions into a 1.7 mL microfuge tube.

All preliminary cDNA cleanup testing was performed in a 1.7 mL microfuge tube. Smaller or larger microfuge tubes may also work, but have not been tested.

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

2. Add AMPure according to the chart below:

<i>Reaction Volume (μL)</i>	<i>AMPure Volume (μL)</i>
50	90
100	180
150	270
200	360

The volume of AMPure for a given reaction can be derived from the following equation:

$$1.8 \times (\text{Reaction Volume}) = (\text{Volume of AMPure per reaction})$$

3. Mix the AMPure and the sample thoroughly. Let the tube incubate at room temperature for 5 minutes before proceeding to the next step.

This step binds the DNA to the magnetic beads. Mix by pipette pipette mixing. Agencourt recommends at least 10 pipette mixes. The color of the mixture should appear homogenous after mixing.

4. Place the microfuge tube into a tube-rack magnet to separate beads from solution.

The recommended separation time is 10 minutes. The solution should be clear before proceeding to the next step. Separation times depend on reaction size volumes; larger reactions may require a longer separation time.

5. Aspirate the cleared solution from the microfuge tube and discard it.

This step should be performed with the tube situated in the rack. Care should be taken not to disturb the separated magnetic beads. You may need to leave a few microliters of the supernatant behind to avoid aspirating out some of the beads.

6. Dispense 400 μL of 70% ethanol into the microfuge tube. Incubate for 30 seconds. Aspirate the wash solution and discard it. Repeat.

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

It is important to perform these steps with the tube situated in the rack. The volume of ethanol could be varied, as long the ethanol level is high enough within the microfuge tube to cover all the beads. The wash solution should be applied for at least 30 seconds before removal. During aspiration, care should be taken not to disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well or it may take a long time to dry.

7. Place the microfuge tube on the bench top to air-dry. Be sure to allow the tube to dry completely.

The tube should be left to air-dry for 25 minutes on a bench top to allow residual ethanol to evaporate completely. Alternatively the tube can be incubated at 37°C for faster evaporation. After the drying is complete, the tube may be stored dry at 4°C or -20°C. Longer drying time may be required for microarraying. Elute your sample just prior to use.

8. Add 20 µl (or more) of elution buffer to the tube.

If the level of elution buffer is not high enough to reach the dried beads, you may have to use a pipette to resuspend the beads from the side of the tube. Let the beads incubate in elution buffer (away from the magnet) for 5 minutes to ensure complete elution. 10 mM Tris-Acetate pH = 8.0, reagent grade water, or TE buffer may be used for elution.

9. Transfer the sample away from the beads.

Place the tube back in the magnet rack for 15 minutes. Carefully aspirate the cleared sample and dispense into a clean microfuge tube. Be careful not to disrupt the beads during the transfer. The 15 minute separation time allows for maximum recovery of sample volume while reducing the risk of bead carry-over.

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)
--------------------------	------------------------------

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

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 μ L within the well of 384 well plates (14 μ L reaction + 25 μ L Agencourt AMPure = 39 μ L).

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 μ L 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

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

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, DiH₂O, 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>

LIMITED USE LABEL LICENSE

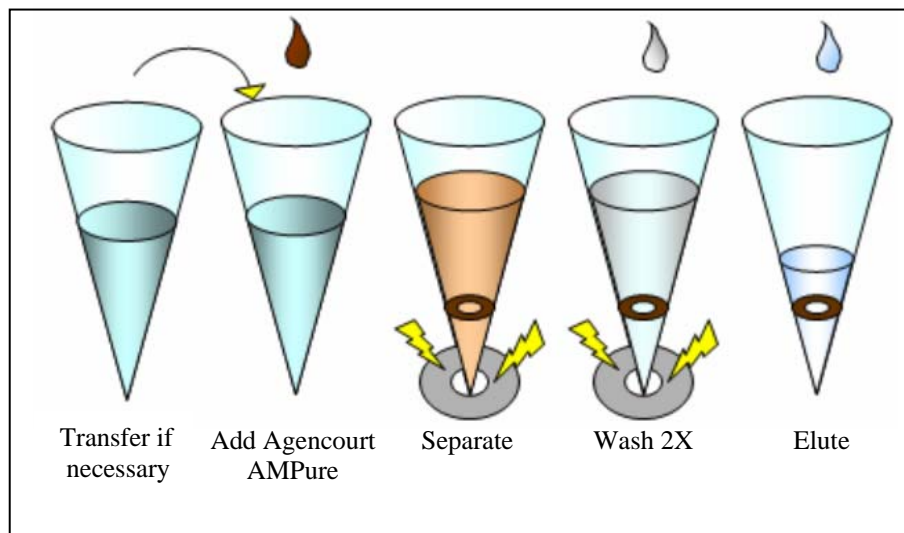
This product is covered by at least one or more claims of US patents Nos. 5,898,071, 5,705,628, and/or 6,534,262, which are exclusively licensed to Agencourt Bioscience Corporation. This product is sold strictly for the use of the buyer and the buyer is not authorized to transfer this product [or any materials made using this product] to any third party.

© 2006 Agencourt Bioscience Corp. All rights reserved.

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

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 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.
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.



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



Innovate Automate
SIMPLIFY