

Instructions For Use

Agencourt AMPure XP

PCR Purification

B37419AA
August 2013



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Agencourt AMPure XP Information For Use Guide
PCR Purification

PN B37419AA (August 2013)

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Revision History

Initial Issue, 08/2013

Agencourt AMPure XP Information For Use version B37419AA

Safety Notice

Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Alerts for Warning, Caution, Important, and Note

WARNING

The signal word WARNING is displayed in an orange signal panel and the associated text (in this example the definition of WARNING) is in bold-face.



WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

In this document the signal word WARNING is only used to indicate the possibility of personal injury. It is not used to indicate the possibility of erroneous data.

CAUTION

The signal word CAUTION is displayed in a yellow signal panel and the associated text (in this example the definition of CAUTION is in bold-face as shown below.



CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

In this document the signal word CAUTION is used to indicate the possibility of damage to the instrument.

IMPORTANT

The signal word IMPORTANT is in bold-face and the associated text (in this example the definition of IMPORTANT) is indented if it wraps.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

The signal word IMPORTANT is used to draw attention to information that is critical for the successful completion of a procedure and/or operation of the instrument.

NOTE

The signal word NOTE is in bold-face and associated text (in this example the definition of NOTE) is indented if it wraps.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

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About This Manual

The information in this manual is organized as follows:

Agencourt AMPure XP PCR Purification

Provides an overview of the Agencourt AMPure XP PCR* purification process, materials such as consumables and reagents needed, and the procedure for either a 96 well format or a 384 well format.

Troubleshooting

Solutions to PCR purification problems such as low yield, low recovery, and incomplete purification.

Frequently Asked Questions

Answers to questions that are commonly asked about the Agencourt AMPure XP PCR purification process.

Abbreviations

Defines most of the abbreviations that are used in this manual.

Glossary

Provides definitions for terms used throughout this manual.

Quick Reference

Quick reference for using the [96 Well Format Procedure](#) for PCR purification.

Intended Use

Agencourt AMPure XP is intended for molecular biology research applications. It is not intended or validated for use in the diagnosis of disease or other conditions.

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

Warranty Disclaimer

Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to the method, including but not limited to warranties of fitness for a particular purpose or merchantability or that the method is non-infringing. All other warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter.

Conventions Used

This manual uses the following conventions:

- Links to the internet or to information in another part of the document are in blue. To access the linked information, select the blue text.

Technical Support

For questions regarding this protocol, call Technical Support for Beckman Coulter at 1-800-369-0333 or contact Technical support by e-mail using: reagentsupport@beckman.com

Agencourt AMPure XP PCR Purification

Introduction

Please refer to <http://www.beckmancoulter.com> for updated protocols and refer to MSDS instructions <https://www.beckmancoulter.com/wsrportal/page/msdsDownloadTab> when handling or shipping reagents.

The Agencourt AMPure XP PCR* purification system utilizes Beckman Coulter's solid-phase reversible immobilization (SPRI) paramagnetic bead technology for high-throughput purification of PCR amplicons. Agencourt AMPure XP utilizes an optimized buffer to selectively bind DNA fragments 100 bp and larger to paramagnetic beads. Excess primers, nucleotides, salts, and enzymes can be removed using a simple washing procedure. The result is a more purified PCR product.

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

- PCR
- Sequencing
- Genotyping
- Fragment Analysis
- Primer Walking
- Cloning

NOTE AMPure XP is supported for SNP 6.0 by Affymetrix. For inquiries regarding this application, please call 1-888-362-2447 or your local Affymetrix support organization.

AMPure XP is used in several Next Generation Sequencing Applications and is supported by the organizations which developed these protocols.

This protocol is intended for the following Products

- A63880 AMPure XP 5 mL
- A63881 AMPure XP 60 mL
- A63882 AMPure XP 450 mL

The AMPure XP purification procedure is highly amenable to Beckman Coulter Biomek automation platforms because it utilizes magnetic separation and requires no centrifugation or vacuum filtration. More information on automating Agencourt AMPure XP can be found at the Beckman

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

Coulter website at: <http://www.beckmancoulter.com/>. Once on the site, search (use magnifying glass icon) for AMPure XP.

Material Supplied

- Agencourt AMPure XP
 - Store at 4°C upon arrival, for up to 18 months
 - Shake the reagent well before use. It should appear homogenous and consistent in color

Specifications

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

Table 1 Available Agencourt AMPure XP

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

Table 2 Number of PCR Reactions Purified with 96 Well

PCR Reaction Volumes 96 Well Format (µL)	Product Number A63880	Product Number A63881	Product Number 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

Table 3 Number of PCR Reactions Purified with 384 Well

PCR Reaction Volumes 384 Well Format (µL)	Product Number A63880	Product Number A63881	Product Number 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 by the User

Consumables and Hardware:

Reaction Plate

For 96 well format — 96 well thermal cycling plate (for example: ABgene product # AB-0800; AB-2800 or AB-1400 <http://www.fishersci.com>), or 300 µL round bottom microtiter plate (Costar # 07-200-105; <http://www.fishersci.com>), or 1.2 mL deep well microtiter plate (Product # AB-1127 <http://www.fishersci.com>)

For 384 well format — 384 well (40 µL well capacity) cycling plate (for example: Hard-Shell Bio-Rad PCR plate # HSP-3801 or ABgene product # AB-1111 <http://www.fishersci.com>)

Agencourt SPRIPlate Magnetic Plate

For 96 well format — Agencourt SPRIPlate 96 Ring Super Magnet Plate (Beckman Coulter product # A32782; <https://www.beckmancoulter.com>)

For 384 well format — Agencourt SPRIPlate 384 (Beckman Coulter product # A29165; <https://www.beckmancoulter.com>)

Plate Seals, Adhesive or Heat

- For example: Product # AB-3739; <http://www.fishersci.com>

Liquid Handling Robotics or a Multichannel Hand Pipette

- Recommended

Reagents:

Fresh 70% ethanol

Fresh 70% ethanol should be prepared for optimal results.

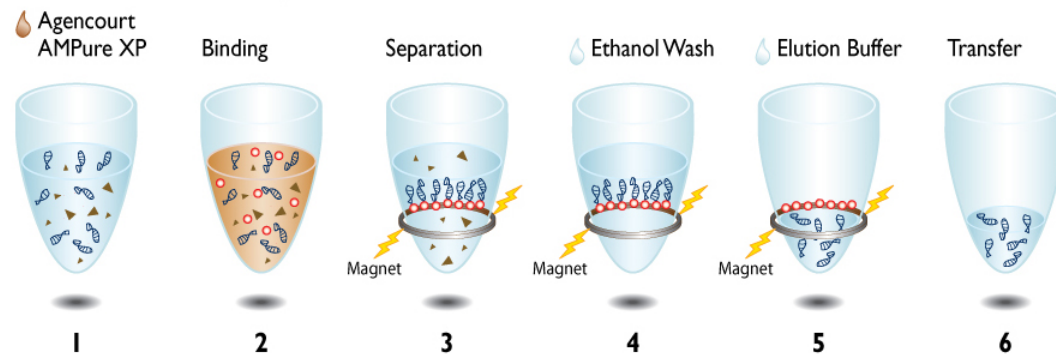
NOTE 70% ethanol is hygroscopic. That is, when opened the ethanol will both evaporate and absorb water over time. Re-use eventually will be at a lower concentration. There is also miscibility involved with ethanol and water. For example, measuring out 70 mL of ethanol and topping off to 100 mL with water will generate ~65% ethanol. Measuring 70 mL ethanol and 30 mL water separately, then combining them will generate ~95 mL of 70% ethanol.

Reagent Grade Water

Either water, TRIS-Acetate (10 mM pH 8.0), or TE Buffer (10 mM Tris-Acetate pH 8.0, 1 mM EDTA) for DNA elution.

PCR Purification Process Overview

Figure 1 Workflow for PCR Purification



The workflow for the PCR purification process is as follows:

1. Add 1.8 μL AMPure XP per 1.0 μL of sample.
2. Bind DNA fragments to paramagnetic beads.
3. Separation of beads + DNA fragments from contaminants.
4. Wash beads + DNA fragments twice with 70% Ethanol to remove contaminants.
5. Elute purified DNA fragments from beads.
6. Transfer to new plate.

A detailed procedure for using a 96 well format to perform PCR purification can be found in the [96 Well Format Procedure](#) section of this manual within [PCR Purification Process Procedure](#).

A detailed procedure for using a 384 well format to perform PCR purification can be found in the [384 Well Format Procedure](#) section of this manual within [PCR Purification Process Procedure](#).

Calculation of Percent Recovery

To gauge percent recovery, analyses of the samples pre-purification and post-purification are necessary. For this process, we recommend either a PicoGreen 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 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.

PCR Purification Process Procedure

For information on automating the Agencourt AMPure XP process, please visit: <http://www.beckmancoulter.com>

96 Well Format Procedure

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 or a 1.2 mL deep-well plate is required.
2. Shake the Agencourt AMPure XP bottle to resuspend any magnetic particles that may have settled. Then add Agencourt AMPure XP according to the sample reaction volume shown in Table 4.

Table 4 AMPure XP to Sample Reaction Volume Chart

Sample 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:

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

3. This step binds DNA fragments 100 bp and larger to the magnetic beads. Pipette mixing is preferable to vortexing as it tends to be more reproducible. The color of the mixture should appear homogenous after mixing:
-- Mix reagent and sample thoroughly by pipette mixing 10 times. Let the mixed samples incubate for 5 minutes at room temperature for maximum recovery.
 4. Place the reaction plate onto an Agencourt SPRIPlate 96 Super Magnet Plate for 2 minutes to separate beads from the solution.
- IMPORTANT** Wait for the solution to clear before proceeding to the next step.
5. This step must be performed while the reaction plate is situated on the Agencourt SPRIPlate 96 Super Magnet Plate:
-- Aspirate the cleared solution from the reaction plate and discard. Leave 5 µL of supernatant behind, otherwise beads are drawn out with the supernatant.

IMPORTANT Do not disturb the ring of separated magnetic beads.

IMPORTANT Perform the next step with the reaction plate situated on an Agencourt SPRIPlate 96 Super Magnet Plate. Do not disturb the separated magnetic beads. Also, be sure to remove all of the ethanol from the bottom of the well.

6. Dispense 200 μ L of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate out the ethanol and discard.

NOTE If the total volume of sample plus reagent exceeds 200 μ L, then use a wash volume of at least the volume of sample plus reagent.

Repeat for a total of two washes.

The beads are not drawn out easily when in alcohol, so it is not necessary to leave any supernatant behind.

NOTE A dry time is optional to ensure all traces of Ethanol are removed. For fragments 10 kb and larger, do not over dry the bead ring (bead ring appears cracked if over dried) as this will significantly decrease elution efficiency.

7. Remove the reaction plate from the magnet plate, and then add 40 μ L of elution buffer to each well of the reaction plate and pipette mix 10 times. Incubate for 2 minutes.

The liquid level will be high enough to contact the magnetic beads at a 40 μ L elution volume. A greater volume of elution buffer can be used, but using less than 40 μ L 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.

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 eluate to a new plate.

NOTE Bead carryover into the final plate is usually not a cause for concern. The samples can be stored in the freezer with beads and the beads are inert in downstream enzymatic reactions. If bead carryover must be limited for any reason, 2 μ L – 5 μ L of eluate can be left behind in the original plate. In addition, a second transfer away from the beads is optional. To do so, place the final plate containing beads and eluate onto the magnet for 1 minute to separate the beads. Transfer the eluate into another clean plate.

384 Well Format Procedure

1. Shake the Agencourt AMPure XP bottle to resuspend any magnetic particles that may have settled. Then add Agencourt AMPure XP according to the sample reaction volume shown in Table 5.

NOTE Due to the total volume of sample plus reagent, it is not possible to purify reactions larger than 14 μL within the well of a 384 well PCR plate as shown in the following equation:

$$(14 \mu\text{L reaction} + 25 \mu\text{L Agencourt AMPure XP} = 39 \mu\text{L})$$

Table 5 AMPure XP to Sample Reaction Volume Chart

Sample Reaction Volume (μL)	AMPure XP 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:

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

2. This step binds DNA fragments 100 bp and larger to the magnetic beads. Pipette mixing is preferable to vortexing as it tends to be more reproducible. The color of the mixture should appear homogenous after mixing:
 - 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.
3. Place the reaction plate onto an Agencourt SPRIPlate 384 for 2 minutes to separate beads from the solution.

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

4. This step must be performed while the purification plate is situated on the Agencourt SPRIPlate 384:
 - Aspirate the cleared supernatant from the reaction plate and discard. Leave a few μL of supernatant behind, otherwise beads are drawn out with the supernatant.

IMPORTANT Do not touch the magnetic beads, which have formed a spot on the side of the well.

IMPORTANT Perform the next step with the reaction plate situated on an Agencourt SPRIPlate 384 Post Magnet Plate. Do not disturb the separated magnetic beads. Also, be sure to remove all of the ethanol from the bottom of the well

5. 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.

The beads are not drawn out easily when in alcohol, so it is not necessary to leave any supernatant behind.

NOTE A dry time is optional to ensure all traces of Ethanol are removed. For fragments 10 kb and larger, do not over dry the bead pellets (bead pellets appear cracked if over dried) as this will significantly decrease elution efficiency.

6. Remove the reaction plate from the magnet plate, and then add 30 μ L of elution buffer to each well and pipette mix 10 times. Incubate for 2 minutes.

A 30 μ L 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 μ L requires extra mixing (to ensure the liquid comes into contact with the beads) and may not fully elute the entire product.

7. Place the reaction plate onto an Agencourt SPRIPlate 384 for 1 minute to separate beads from the solution.
8. Transfer the eluate to a new plate.

NOTE Bead carryover into the final plate is usually not a cause for concern. The samples can be stored in the freezer with beads and the beads are inert in downstream enzymatic reactions. If bead carryover must be limited for any reason, 2 μ L – 5 μ L of eluate can be left behind in the original plate. In addition, a second transfer away from the beads is optional. To do so, place the final plate containing beads and eluate onto the magnet for 1 minute to separate the beads. Transfer the eluate into another clean plate.

PCR Purification Problems and Solutions

Solutions to PCR purification problems such as low yield, low recovery, and incomplete purification are given in the charts below.

Low Yield / Recovery Problems	Possible Solutions
Recovery was measured by Spectrophotometry Absorbance	Pre-purification unincorporated primers and nucleotides will contribute to the absorbance, so the un-purified PCR reaction concentration will appear higher than it actually is. This causes the recovery to appear lower than it actually is. Run an equal portion of the un-purified PCR reaction alongside the purified PCR reaction (for example $\frac{1}{4}$ of the un-purified PCR product and $\frac{1}{4}$ of the eluate) on an agarose gel to double check the recovery measurement, or use a PicoGreen assay.
Bead Loss	If beads get aspirated into tips during supernatant removal, the nucleic acid bound to these beads will also be lost. Aspirate slowly and remove as much of the first supernatant as possible without disturbing the bead ring / pellet. If beads are aspirated by accident, dispense everything back into the well, allow the beads to resettle before aspirating again. Try aspirating slower or with a finer pipette. Low concentrated samples will be more susceptible to bead loss since there is less sample keeping the beads in place. Low volume samples will be more susceptible to bead loss since the beads may not reach the level of the magnet in the well.
Fragment size incompatible	AMPure XP was made for PCR purification of PCR products. For size selection applications consider using SPRIselect. More information on SPRIselect can be found on the web at: www.spriselect.com/
Insufficient Mixing	Mixing thoroughly during the initial bind mix and elution mix is critical. Mix with a tip volume just slightly below the total well volume. At elution, the minimum elution volume (40 μ L for 96 well format and 15 μ L for 384 well format) is needed to ensure the beads get sufficiently resuspended. Incubation times should also be maintained to ensure the nucleic acid has enough time to bind or dissociate with the beads. Vortexing during binding can be inefficient because of the viscosity of the sample.

Low Yield / Recovery Problems	Possible Solutions
Large Reaction Volume	Large volume reactions can benefit from an extended binding and separation time. Increase binding time to 10 minutes and ensure all beads are separated before removing the supernatant.
Ethanol	Ethanol must be at least 70%. When diluting 100% ethanol to 70%, ensure that water and ethanol are measured SEPARATELY before combining due to the miscibility of ethanol. Topping off ethanol with water to dilute will lead to a lower concentration than intended. Stock ethanol can also absorb water from the atmosphere over time leading to a lower concentration. Ensure stock ethanol remains tightly capped when not in use.
Low Elution Volume	A small elution volume leads to a decrease in recovery. This is because a small amount of elution buffer always stays behind coating the beads. This volume is dependent on the well shape and the amount of beads in the well, so a smaller elution volume will lead to a higher percentage of eluate staying behind.
Large fragments dried completely onto the beads	Fragments exceeding a few kb can bind very tightly to the beads if the samples dry out and they can be difficult to elute off. Remove the last ethanol wash as completely as possible, but add the elution buffer right away and mix the samples. Ethanol will continue to evaporate out of the well as it sits open during the elution incubation.

Incomplete Purification Problems	Possible Solutions
Primer Carryover	The final concentration of the binding buffer contained in the AMPure XP reagent determines the size fragments binding to the beads. AMPure XP was made to capture PCR products > 100 bp and eliminate primers < 50 bases. Larger primers and primer dimers can bind to the beads. Ensure that the correct volume of reagent is added and that your PCR product has not evaporated to a smaller volume during cycling. Ensure the well is rinsed with ethanol wherever the sample touched the plastic during mixing and binding. If an excess of primer is present during purification, reducing the amount of primer used for the PCR reaction can also be beneficial.
Poor Performance in Downstream Enzymatic Reactions	If your downstream enzymatic reaction is extremely sensitive to trace ethanol, add a conservative dry step of two minutes. Note that large fragments can bind strongly to the beads and may be difficult to elute off after drying onto the beads completely.

Frequently Asked Questions

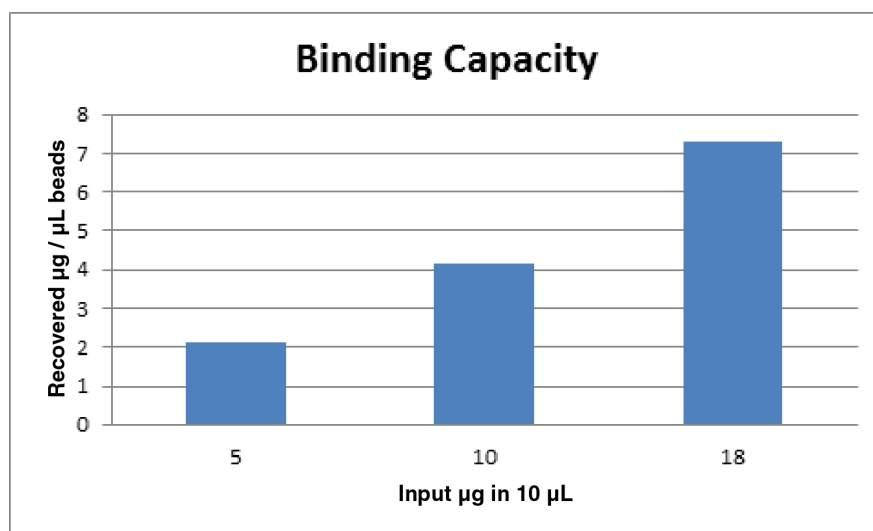
PCR Purification Questions and Answers

Answers to questions that are commonly asked about the Agencourt AMPure XP PCR purification process are provided below.

1. What is the binding capacity of the beads?

The binding capacity of the beads is so high that they cannot be exceeded with any sample type. It is possible to bind at least 7 μg of nucleic acid to 1 μL AMPure XP reagent, however the sample will become so viscous that it is difficult to pipette the sample. Refer to [Figure 2](#).

Figure 2 Bead Binding Capacity



2. The AMPure XP was at room temperature for some time, can I still use it?

AMPure XP is manufactured and tested for the storage temperature indicated on the bottle and we can guarantee performance only at that temperature.

3. The AMPure XP was accidentally frozen, can I still use it?

AMPure XP is manufactured and tested for the storage temperature indicated on the bottle and we can guarantee performance only at that temperature.

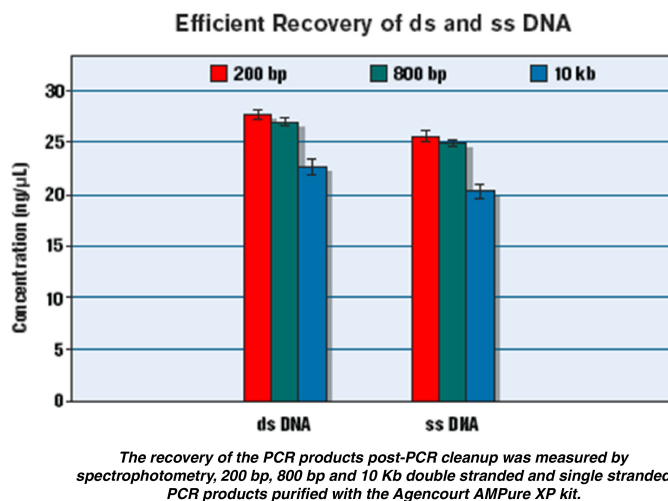
4. Can I replace the AMPure XP in the SPRIWorks HT kit PN B05451 with AMPure XP PNs A63880, A63881 or A63882?

It is not recommended. The AMPure XP in the SPRIworks HT kit is tested for Next Generation Sequencing applications, other lots of AMPure XP are not.

5. Can AMPure XP bind double-stranded nucleic acids and single-stranded nucleic acids?

Yes, AMPure XP will bind single stranded and double stranded nucleic acids. Refer to Figure 3.

Figure 3 Binding of Nucleic Acids



6. Can I purify gDNA with AMPure XP?

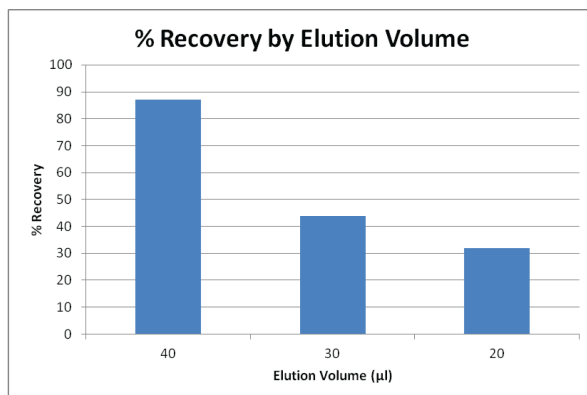
Yes, follow the normal protocol and make sure that the beads never dry out, because large fragments bind so tightly to the beads that they can be difficult to elute off.

7. Can I elute my sample in less than 40 μL in 96 well format?

Yes, but the recovery will decrease with a decrease in elution volume. In addition, if the beads cannot reach the magnet during the separation it may be necessary to leave a portion of the eluate behind if bead carryover must be avoided. Be sure to resuspend the beads fully in the elution buffer. An example of decrease in recovery with reduction in elution volume is shown in Figure 4.

NOTE If there is a need for elution volumes under 40 μL, then you may want to try the Low Elution Magnet Plate (LE Magnet Plate, Part # A000350). Substitute the LE Magnet plate for the Super Magnet Plate (A001322) during the final elution step. Separation time will need to be increased. For more information on the LE Magnet Plate, visit the manufacturer's website at: <http://www.alpaqua.com>

Figure 4 Elution Volume Decrease in Recovery



8. *Can the AMPure XP protocol be done in tubes?*

Yes, just follow the normal protocol. For 1.5 mL and 2.0 mL tubes we recommend the Agencourt SPRIStand Magnet PN A29182. For 0.2 mL tubes and strip tubes, the Super Magnet Plate PN A32782 can be used. Note, that some tubes have a coating that makes the beads slide off the side of the tube, making supernatant removal steps more challenging.

9. *Can I dry my samples before elution to eliminate trace ethanol?*

A dry step can be incorporated after the removal of the last ethanol wash and before the addition of the elution buffer. For purification of very large fragments, such as gDNA a dry step is not recommended because recovery would be decreased. Fragments up to 40 kb have been known to tolerate a dry step.

10. *Is there an upper size limit for the fragments that can be bound with AMPure XP?*

No, AMPure XP will bind fragments 100 bp and above and exclude primers 50 bases and below. Between 50 and 100 bases there will be some recovery.

11. *What is the recovery with AMPure XP?*

The recovery depends on the fragment size, sample volume, sample concentration, elution volume and some other factors. The under ideal conditions recovery can be up to 90%, but under sub optimal it can be as low as 60%. Refer to [Figure 5](#) and [Figure 6](#).

Figure 5 Recovery Percentage by Input Volume

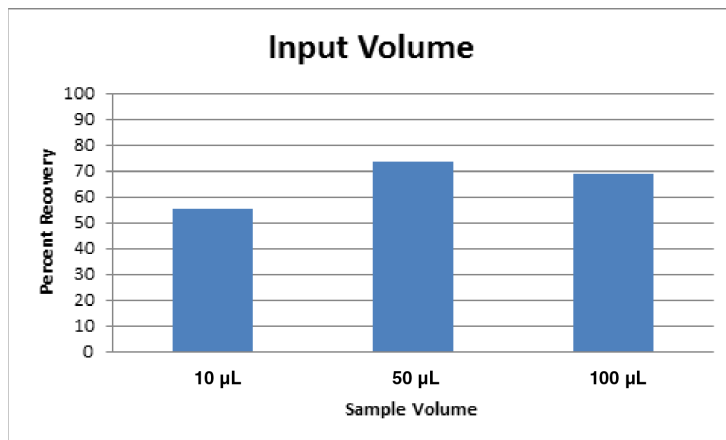
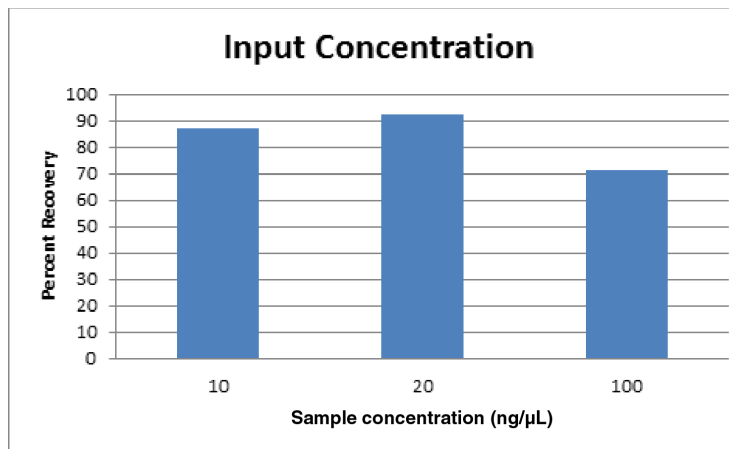


Figure 6 Recovery Percentage by Input Concentration



Abbreviations

μg — microgram

μL — microliter

bp — base pair

DNA — Deoxyribonucleic Acid

gDNA — Genomic Deoxyribonucleic Acid

m — meter

mL — milliliter

mM — millimolar

ng/μL — nanograms per microliter

PCR — Polymerase Chain Reaction

qPCR — quantitative (real-time) PCR

SN — Supernatant

SNP — Single Nucleotide Polymorphism

SPRI — Solid Phase Reversible Immobilization

TE — Tris with Ethylenediaminetetraacetic acid
(10 mM Tris, pH 8, 1 mM EDTA)

Tris — tris(hydroxymethyl)aminomethane (10 mM, pH 8)

Vol — Volume

Glossary

aliquot — a portioning out of precise amounts.

assay — procedure of repeat testing to determine the assigned value for a given lot and level of control.

beads — in SPRI technology, magnetic, uniform microparticles.

dead volume — in an automated system, the amount or volume of a sample or reagent that cannot be picked up by the pipette tip.

elution buffer — buffer which elutes DNA from magnetic particles.

ethanol wash — washes the magnetic beads with 70% ethanol to remove contaminants.

pipetting — Use of a laboratory device for the volumetric measurement and transfer of fluids from one container to another.

protocol — a written procedural method in the design and implementation of experiments.

reservoir — one-well labware receptacle holding liquid to be used in a method.

supernatant — a liquid lying above a solid residue after crystallization, precipitation, centrifugation, or other process.

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Quick Reference for 96 Well Format

Below is a quick reference to the procedure for using a 96 well format to perform PCR purification.

For 96 well format:

- 1** Sample volume X 2.8 > Well volume?
 - Yes, go to step 2.
 - No, go to step 3.

- 2** Transfer sample to a 300 µL round bottom plate or a 1.2 mL deep well plate.

- 3** Shake the Agencourt AMPure XP bottle to fully resuspend magnetic particles.

- 4** Add sample volume 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 (or total volume of reaction plate) 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 20 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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