AGENCOURT® GENFIND™ v2

Blood & Serum Genomic DNA Isolation Kit

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 Genfind™ v2 Blood & Serum DNA Isolation Kit utilizes Agencourt's patented SPRI® paramagnetic bead technology to isolate genomic DNA from fresh or frozen whole blood and serum containing Citrate, EDTA, or Heparin anticoagulants. The protocol can be performed in both 96-well and single tube formats. Purification begins by the addition of a lysis buffer and Proteinase K to rupture cell membranes and digest protein. DNA is then immobilized on magnetic particles by the addition of a magnetic binding reagent. This differential binding allows the DNA to be easily separated from contaminants using a magnetic field. Contaminants can then be rinsed away using a simple washing procedure, leaving the genomic DNA ready for elution from the magnetic particles. The 96-well plate format procedure is highly amenable to automation since it utilizes magnetic separation, thus eliminating the need for vacuum filtration or centrifugation.

Genomic DNA from the Agencourt Genfind™ v2 Kit can be used in:

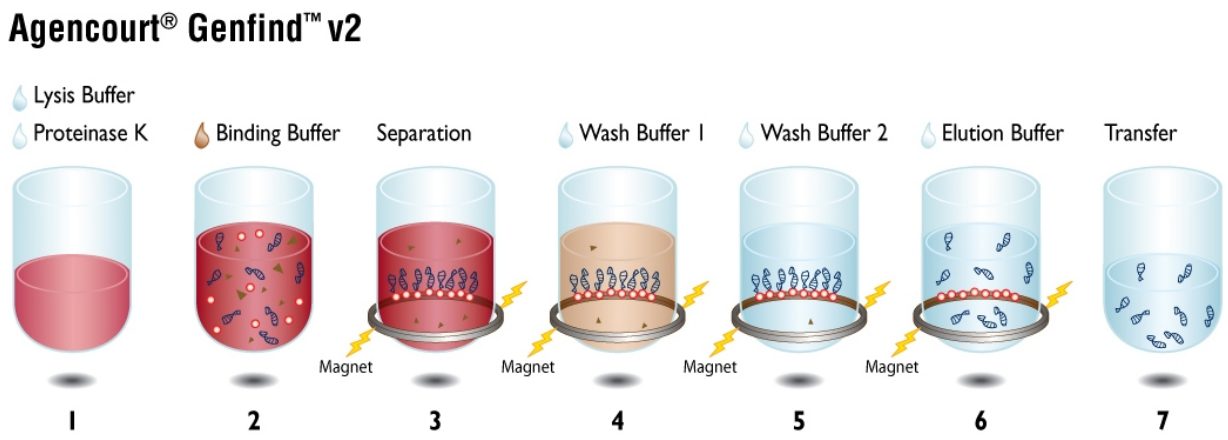
- Agarose gel analysis
- PCR[1] amplification
- Restriction enzyme digestion
- Human identity testing
- Membrane hybridizations (e.g., Southern and dot/slot blots).
- AFLP, RFLP, RAPD, microsatellite and SNP analyses (for genotyping, fingerprinting, etc.)

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

Warnings and Precautions

1. Agencourt Bioscience Corp. kits are intended ***For Laboratory Use*** only.
2. The U.S. Centers for Disease Control, the Food and Drug Administration, and the American Hospital Association recommend applying "universal precautions" when handling blood and body fluids to protect health care and laboratory workers. Under universal precautions, blood and body fluids are considered potentially infectious for blood-borne pathogens. It is recommended that workers protect themselves from contact with the fluids by using suitable barrier protection which includes gloves. Purified DNA may contain blood-borne pathogens, so effective barrier protections should be used throughout all stages of this procedure.

Process Overview:



1. Lyse whole blood or serum in Lysis Buffer and Proteinase K
2. Bind genomic DNA to paramagnetic beads
3. Separate beads from contaminants
4. Wash the magnetic beads with Wash Buffer 1 to remove contaminants
5. Wash the magnetic beads with Wash Buffer 2 to remove contaminants
6. Elute DNA from magnetic particles
7. Transfer to new plate

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

Kit Specifications:

	Tube Kit (50 preps) A41501 and A41499	Plate Kit (4x96 preps) A41497 and A41500
200 µL Sample	100 preps	384 preps
400 µL Sample (Tube format only)	50 preps	192 preps

Reagents supplied in the kit:

Reagent	Description	Storage Condition
Lysis Buffer	Blood/Serum Lysis (clear)	Room Temperature
Proteinase K	Lyophilized Enzyme (1 mL tubes)	-20°C
Proteinase K Buffer	Proteinase K Buffer (clear)	Room Temperature
Binding Buffer	Magnetic Solution (in light protection bottle)	4°C DO NOT FREEZE
Wash Buffer 1	DNA Wash Buffer 1 (clear)	Room Temperature DO NOT REFRIGERATE DO NOT HEAT
Wash Buffer 2	DNA Wash Buffer 2 (clear)	Room Temperature

The reagents have a shelf life of 6 months if stored as directed.

Consumables and Hardware:

- **Magnetic Separator:**
  For 96 well format: Agencourt SPRIPlate 96 Ring Super Magnet plate [Agencourt #000322; http://www.agencourt.com/ ; Beckman Coulter A32782 www.beckmancoulter.com/]
  For single tube format: Agencourt SPRIStand magnetic tube rack [Agencourt #001139; http://www.agencourt.com/; Beckman Coulter A29182 www.beckmancoulter.com/]
- **Reaction Plate:**
  For 96 well format: 96 well 1.2 mL magnet compatible deepwell block [ABGene #AB-1127; http://www.abgene.com]
  For single tube format: 2.0 mL microcentrifuge tubes; [ABGene #T5022; http://www.abgene.com]
  Pipettes: p20 and p1000 with aerosol barrier tips
  Seals (for 96 well format): Adhesive PCR film [ABGene Cat # AB-0558 http://www.abgene.com

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Reagents:

- 100% Ethanol
- Elution buffer TE Buffer (10 mM Tris Cl, 1 mM EDTA, pH8) for long term storage or 10 mM Tris, pH 8 (1M Tris-HCl, pH 8: American Bioanalytical product # AB14043 http://www.americanbio.com/) or Reagent Grade Water (Ambion product # 9932; http://www.ambion.com)

Plate Purification Procedure (For up to 200 µL of Blood/Serum):

Starting Material: Agencourt Genfind™ v2 Blood & Serum Kit can be used with fresh or frozen whole blood containing Citrate, EDTA, or Heparin anticoagulants. Frozen samples should be thawed at room temperature or 37°C, then mixed well before beginning the protocol.

The 96 well plate format allows purification up to 200 µL of blood/serum per well. The protocol below lists reagent additions based on a 200 µL starting volume. The reagent volumes should be scaled linearly if starting with smaller sample volumes.

Please note that modifications to this protocol were necessary for automated processing with the Agencourt Genfind™ v2 methods for Biomek FX 96 and NX Span-8.

Agencourt strongly recommends using aerosol-barrier (filter) pipette tips when performing the Agencourt Genfind™ v2 purification.

1. **For each new kit, assemble Proteinase K Solution and Wash Buffer 2 according to the following chart:**

	Proteinase K Solution	Wash Buffer 2
	Volume of PK Buffer to add to Proteinase K per tube	**Volume of 100% ethanol to add to Wash Buffer 2 concentrate**
Tube Kits (A41499 and A41501)	1 mL	105 mL
Plate Kits (A41497 and A41500)	1 mL	375 mL
Storage condition once prepared	-20°C	Room temperature

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2. **Mix the blood gently by inverting the stock tube several times. Tipmixing or vortexing is not recommended. Aliquot 200 µL of fresh or frozen blood into the magnet compatible 1.2 mL 96-well plate (AB-1127 from ABGene).**

   Mixing blood thoroughly before aliquoting helps to increase yield. For a plate to be 'magnet compatible', the bottom of each well should directly contact each ring magnet. Ideally, the wells should have a round bottom without any plastic extrusions. ABGene's AB-1127 has been tested extensively by Agencourt.

3. **Add 400 µL (2 x sample volume) Lysis Buffer and 9 µL (0.045 x sample volume) of 96 µg/µL Proteinase K to the samples. Gently pipette tipmix 10 times or until well mixed.**

   Use Proteinase K with a concentration of 96 µg/µL as prepared in step 1. When lysing the samples, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles.

4. **Incubate the samples at 37°C for 10 minutes, or at room temperature for 30 minutes.**

   For lysis at 37°C, samples can be sealed and placed in a water bath.

5. **IMPORTANT: Invert the Binding Buffer bottle 20 times to ensure complete resuspension of magnetic particles before using. Add 300 µL (1.5 x sample volume) Binding Buffer to the samples and gently pipette tipmix 10 times or until well mixed.**

   During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

6. **Incubate the plate at room temperature for 5 minutes to bind.**

7. **Place the sample plate on an Agencourt SPRIPlate 96 Ring Super magnet for 15 minutes to separate.**

   The solution will be very dark in color and it will be difficult to see the ring of beads form at the bottom of the plate. As long as the samples have been allowed to separate for the specified time, it can be assumed that a complete ring has formed.

8. **Aspirate off the supernatant and discard while the plate is situated on the magnet.**

   Due to the large volume of supernatant, this step may require multiple aspirations to remove all the liquid. It will be difficult to see the ring of beads at the bottom of the well until the liquid level gets low. When aspirating, place the pipette at the center of the well to avoid disturbing the magnetic beads.

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9. **Take the plate off the magnet. Add 800 µL (4 x sample volume) of Wash Buffer 1 and pipette tipmix 10 times (with a 1mL pipette set to 0.8 mL) or until the magnetic beads are resuspended from the bottom of the well.**

   Pipette tipmix until most of the magnetic beads are back in suspension. A few beads may still stick to the bottom of the well, and some of the resuspended beads may form clumps. If a white precipitate has formed in the Wash Buffer 1 prior to use, gently shake or stir at room temperature until the solids dissolve. DO NOT HEAT to re-dissolve.

10. **Place the plate back on the magnet for 10 minutes, or until the solution clears.**

    The supernatant may be brownish in color due to residual blood components.

11. **Aspirate and discard the supernatant while the plate is situated on the magnet.**

    Avoid disrupting the ring of beads.

12. **Repeat steps 9 through 11 for a second wash with the Wash Buffer 1.**

    During the second wash, the beads will not clump as much as in the first wash. Mixing well is critical at this step as Wash Buffer 1 helps to rinse away digested protein. Incomplete resuspension may cause beads to clump together during the final elution step, which can make transfer of the eluant difficult.

13. **Take the plate off the magnet. Add 500 µL (2.5 x sample volume) of Wash Buffer 2 as prepared in step 1 and pipette tipmix 10 times (with a 1mL pipette set to 0.5 mL) or until the magnetic beads are completely resuspended from the bottom of the well.**

14. **Place the plate back on the magnet for 6 minutes, or until the solution clears.**

15. **Aspirate and discard the supernatant while the plate is situated on the magnet.**

16. **Repeat steps 13-16 for a total of two Wash Buffer 2 washes.**

17. **Remove as much of the final wash buffer as possible. For BLOOD: Add 200µL of elution buffer to each sample to elute. For SERUM: Add 40 µL (independent of sample volume) of elution buffer to each sample to elute.**

    Use smaller elution volumes if higher DNA concentrations are desired. Drying samples is not suggested for this protocol as over-drying the DNA onto the beads makes it difficult to fully elute the samples. Use a smaller pipette to remove any remaining visible liquid, if necessary. If the beads appear very wet, a conservative dry time of 5 minutes at room temperature could be used. Elute the samples before the beads appear cracked.

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18. **Remove the plate from the magnet and resuspend the beads by gently pipette tipmixing. Incubate the plate for 2 minutes at room temperature, and then pipette tipmix again to complete the elution.**

19. **Place the plate back on the magnet for 10 minutes, or until the supernatant clears. Transfer the supernatant to a clean plate or clean tubes leaving approximately 25 µL behind.**

    For serum there is no need to leave any eluant behind. If beads are being aspirated during the transfer, dispense the sample back into the well and let the plate sit for an additional 10 minutes to better compact the bead ring. During the transfer, place the pipette tip in the center of the bead ring and aspirate slowly.

Note for Serum: Use at least 2 µL of the 40 µL eluant per PCR reaction for best results.

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Tube Purification Procedure (For up to 400 µL of Blood/Serum):

Starting Material: Agencourt Genfind™ v2 Blood & Serum can be used with fresh or frozen whole blood containing Citrate, EDTA, or Heparin anticoagulants. Frozen samples should be thawed at room temperature or 37°C then mixed well before beginning the protocol.

The tube format can purify up to 400 µL of blood per 2 mL tube. The protocol below lists reagent additions based on a 400 µL starting volume. The reagent volumes can be scaled linearly if starting with smaller or larger sample volumes.

If you are purifying 400 µL of blood/serum, be sure to use a 2mL microcentrifuge tube (i.e. ABGene #T5022). Smaller tubes will not be able to accommodate the reagent volumes necessary for purification of 400 µL blood/serum. If only 1.7 mL microcentrifuge tubes are available, decrease the starting blood/serum volume to 300 µL.

Agencourt strongly recommends using aerosol-barrier (filter) pipette tips when performing the Agencourt Genfind™ v2 purification.

1. **For each new kit, assemble Proteinase K Solution and Wash Buffer 2 according to the following chart:**

	Proteinase K Solution	Wash Buffer 2
	Volume of PK Buffer to add to Proteinase K per tube	**Volume of 100% ethanol to add to Wash Buffer 2 concentrate**
Tube Kits (A41499 and A41501)	1 mL	105 mL
Plate Kits (A41497 and A41500)	1 mL	375 mL
Storage condition once prepared	-20°C	Room temperature

2. **Mix the blood gently by inverting the stock tube several times. Tipmixing or vortexing is not recommended. Aliquot 400 µL of fresh or frozen blood into a 2 mL microcentrifuge tube.**

   Mixing blood thoroughly before aliquoting helps to increase yield.

3. **Add 800 µL (2 x sample volume) Lysis Buffer and 18 µL (0.045 x sample volume) of 96 µg/µL Proteinase K to the samples. Gently pipette tipmix 10 times or until well mixed.**

   Use Proteinase K with a concentration of 96 µg/µl as prepared in step 1. When lysing the samples, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles.

   Add the 800 µL in two portions to avoid wetting the filter in the filter tip. When lysing the samples, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles.

4. **Incubate the samples at 37°C for 10 minutes, or at room temperature for 30 minutes.**

   For lysis at 37°C, samples can be placed in a water bath or a thermal cycler that is compatible with microcentrifuge tubes.

5. **IMPORTANT: Invert the Binding Buffer bottle 20 times to ensure complete resuspension of magnetic particles before using. Add 600 µL (1.5 x sample volume) Binding Buffer to the samples and using a p1000 pipette gently pipette tipmix 10 times or until well mixed.**

   During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the magnet, thus decreasing yield.

6. **Leave the sample tube on bench for 5 minutes to bind.**

7. **Place the tube on the Agencourt SPRIstand magnet for 15 minutes to separate.**

   The solution will be very dark in color and it will be difficult to see the beads separate on the side of the tube. As long as the samples have been allowed to separate for the specified time, it can be assumed that the beads have separated completely.

8. **Aspirate off the supernatant and discard while the tube is situated on the magnet.**

   Due to the large volume of supernatant, this step may require multiple aspirations to remove all the liquid. It will be difficult to see the beads on the side of the tube until the liquid level gets low. When aspirating, place the pipette on the side the tube opposite the magnet to avoid touching the magnetic beads.

9. **<u>Take the tube off the magnet.</u> Add 1.6 mL (4 x sample volume) of Wash Buffer 1 and pipette tipmix 10 times (with a 1mL pipette set to 0.8 mL) or until the magnetic beads are resuspended from the side of the tube.**

   Mix until the magnetic beads are back in suspension. A few beads may still stick to the side of the tube, and some of the resuspended beads may form clumps. If a white precipitate has formed in Wash Buffer 1 prior to use, gently shake or stir at room temperature until the solids dissolve. DO NOT HEAT to re-dissolve.

10. **Place the tube back on the magnet for 2 minutes, or until the solution clears.**

    The supernatant may be brownish in color due to residual blood components.

11. **Aspirate and discard the supernatant while the tube is situated on the magnet.**

12. **Repeat steps 9 through 11 for a second wash with Wash Buffer 1.**

    During the second wash, the beads will not clump as much as in the first wash. Mixing well is critical at this step as Wash Buffer 1 helps to rinse away digested protein. Incomplete resuspension may cause beads to clump together during the final elution step which can make transfer of the eluant difficult.

13. **<u>Take the tube off the magnet.</u> Add 1 mL (2.5 x sample volume) of Wash Buffer 2 as prepared in step 1 and pipette tipmix 10 times (with a 1 mL pipette set to 0.8 mL) or until the magnetic beads are completely resuspended from the side of the tube.**

14. **Place the tube back on the magnet for 6 minutes, or until the solution clears.**

15. **Aspirate and discard the supernatant while the tube is situated on the magnet.**

16. **Repeat steps 13- 16 for a total of two Wash Buffer 2 washes.**

17. **Remove as much of the final wash buffer as possible. For BLOOD: Add 400µL of elution buffer to each sample to elute. For SERUM: Add 40 µL (independent of sample volume) of elution buffer to each sample to elute.**

    Use smaller elution volumes if higher DNA concentrations are desired. Drying samples is not suggested for this protocol as over-drying the DNA onto the beads makes it difficult to fully elute the samples. Use a smaller pipette to remove any remaining visible liquid, if necessary. If the beads appear very wet, a conservative dry time of 5 minutes at room temperature could be used. Elute the samples before the beads appear cracked.

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18. **Resuspend beads off the magnet by gently tipmixing. Incubate the tube for 2 minutes at room temperature, then tipmix again to complete the elution.**

19. **Place the tube back on the magnet for 5 minutes, or until the supernatant clears. Transfer the supernatant to a clean plate or clean tubes for storage (-20°C).**

    If beads are being aspirated during the transfer, dispense the sample back into the tube and let the tube incubate for an additional 10 minutes to better compact the bead pellet. During the transfer, place the pipette tip on the side of the tube opposite the bead pellet and aspirate slowly.

Note for Serum:	**Use at least 2 µL of the 40 µL eluant per PCR reaction for best results.**

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