AGENCOURT® CLEANSEQ®

Dye-Terminator Removal

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 CleanSEQ Sequencing Reaction Clean-Up system utilizes Agencourt's patented SPRI® paramagnetic bead technology. The protocol can be performed directly in the thermal cycling plate. Agencourt CleanSEQ contains magnetic particles in an optimized binding buffer to selectively capture sequencing extension products. Unincorporated dyes, nucleotides, salts and contaminants are removed using a simple washing procedure. The purification procedure is amenable to a variety of automation platforms since it requires no centrifugation or vacuum filtration. Applications notes for Agencourt CleanSEQ can be found at http://www.agencourt.com/technical/

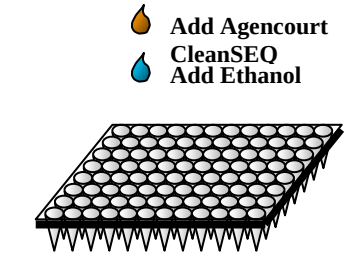
Agencourt has developed two optimized Agencourt CleanSEQ protocols to accommodate various fluorescent-sequencing dye sets (ABI PRISM BigDye Terminator; Beckman Coulter CEQ DTCS and Amersham Biosciences DYEnamic ET Dye Terminators). Please refer to the table of contents for the page on which each protocol begins. During the protocol avoid extensive heat, light or waiting time, as this can lead to degradation of the dyes.

For information about automating Agencourt CleanSEQ, please call Agencourt Bioscience at 1-800-361-7780

Process Overview

The Agencourt CleanSEQ procedure is performed in three stages:

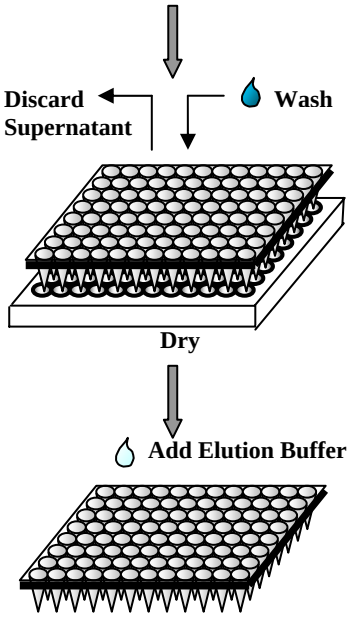
1. Selective binding of sequencing extension products to paramagnetic beads and separation of the beads with a magnetic field
2. Washing of the beads to remove unincorporated dyes, nucleotides, salts, and other contaminants
3. Elution of the purified sequencing product from the paramagnetic beads



Materials Supplied in the Kit:

Agencourt CleanSEQ Magnetic Particle Solution

- Store at 4°C upon arrival, for up to 6 months.
- Mix Agencourt CleanSEQ well before using. The reagent should appear homogenous and consistent in color.
- DO NOT FREEZE.



Materials Supplied by the User:

Consumables & Hardware:

- Agencourt SPRIPlate® magnetic plate:
  For 96 well format: Agencourt SPRIPlate 96R ring magnetic plate [APN 000219]
  For 384 well format: Agencourt SPRIPlate 384 magnetic plate [APN 000222]
- Plate Seals, adhesive or heat. [for example: Abgene product # AB-3739; http://www.abgene.com/]
- An 8 or 12 channel pipette or liquid handling robotics
- For 96 well format: 96 well (300 µL well capacity) round bottom plate [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/]

Reagents:

- Elution Buffer: Reagent grade water, 0.1mM EDTA (pH 8.0), 0.5mM EDTA (pH 8.0) or freshly deionized formamide/Beckman Coulter Sample Loading Solution. The optimal elution buffer will vary depending on dye chemistry and reaction conditions. Please see Table 3 on page 6 for details.

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

- Non-denatured Ethanol (100% or 95% Stock). When you are diluting ethanol stock to the working concentration for Agencourt CleanSEQ, make only as much as you will use in 1-3 days and store it in a tightly capped container. Decreases in ethanol concentration, due to the absorption of water from the surrounding atmosphere, may lead to a loss of product.

Agencourt CleanSEQ for ABI BigDyeTerminator[1] and Beckman Coulter CEQ DTCS

The following table shows the number of ABI BigDye Terminator or Beckman CEQ DTCS reactions that an Agencourt CleanSEQ kit will yield, depending on format.

Table 1

	APN 000121 (8 mL)	*APN 000136 (50 mL)*
96 Well Format	800	5000
384 Well Format	1600	10000

ABI BigDye Terminator/Beckman Coulter CEQ DTCS 96 Well Format:

1. **Gently shake the Agencourt CleanSEQ bottle to resuspend any magnetic particles that may have settled. Add 10µL of Agencourt CleanSEQ to the reaction plate.**

   This step should be performed **before** the plate is placed on the Agencourt SPRIPlate. 10µL of Agencourt CleanSEQ is used regardless of the sequencing reaction volume.

2. **Add 85% ethanol to the reaction plate according to the table below. Pipette mix 7 times, or seal and vortex the reaction plate for 30 seconds.**

   This step should also be performed **before** placing the plate on the Agencourt SPRIPlate. If you are vortexing, use a medium speed (6-8 on a standard mini vortexer) and make sure the suspension is completely homogeneous before continuing. The volume of 85% ethanol needed has been calculated for common sequencing reaction volumes, as shown in Table 2. To determine the volume of ethanol needed for other sequencing reaction volumes use the equation provided below or use the Agencourt Agencourt CleanSEQ calculator at http://www.agencourt.com/technical/reagent_information/cleanseq/

[1] *Agencourt CleanSEQ can be used with all BigDye Terminator versions (1.0, 1.1, 2.0, 3.0, & 3.1).*

Table 2

Agencourt CleanSEQ 96 & 384 for ABI and CEQ Dye Terminators - Volume of ethanol (µL) to add to sequencing reactions of different volumes.		
Volume of Sequencing Reaction (µL)	**Volume of 85% Ethanol (96Well)**	**Volume of 85% Ethanol (384Well)**
5	31	14.3
10	42	21.4
15	52	28.6
20	62	
25	73	

The volume of EtOH to add to each reaction can be determined by the following equation:

$$Vol_{EtOH} = [(Vol_B + Vol_S) \times (C_F/C_E)]/[1 - (C_F/C_E)]$$

Where:

Vol_{EtOH} = volume of ethanol to be used per reaction

Vol_B = volume of Agencourt CleanSEQ used, 10µL for 96 well format

Vol_S = volume of the DNA sample to be cleaned up

C_F = final concentration of ethanol, 57.4% for 96 well format

C_E = concentration of ethanol to be used (Agencourt recommends 85% ethanol)

3. **Place the reaction plate onto an Agencourt SPRIPlate 96R for 3 minutes to separate beads from solution.**

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

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

   This step should be performed while the plate is situated on an Agencourt SPRIPlate 96R. Be careful not to disturb the beads. It is important to completely remove all of the supernatant as it contains excess fluorescent dye and contaminants.

5. **Dispense 100 µL of 85% ethanol to each well and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. For sequencing reactions using**

4 µL or more of BigDye Terminator, a second wash with 100 µL of 85% is necessary to wash out all of the unincorporated dye.

This step should be performed while the plate is situated on an Agencourt SPRIPlate 96R. There is no need to agitate the beads from the side of the well, but it is important to remove all of the ethanol as it contains residual fluorescent dye and contaminants.

6. **Let the reaction plate air-dry for 10 minutes at room temperature.**

The reaction plate does not have to be situated on the Agencourt SPRIPlate 96R during drying. Note that excessive drying can lead to degradation of the incorporated dyes. After removing the final ethanol wash, allow the samples to dry at room temperature for approximately 10 minutes. The plate may then be sealed and stored at 4°C or -20°C. Elute the samples just prior to loading them on the sequencing detector.

7. **Add 40 µL of elution buffer and incubate the plate for 5 minutes at room temperature to elute.**

Elution of the sequencing products from the magnetic beads is rapid and it is not necessary for the beads to go back into solution for complete recovery of the product. For ABI 3100 and 3700 running with POP-5 or POP-6 and the Beckman Coulter CEQ, eluted plates can be loaded directly onto the detector without removal of the beads. For ABI 3730 or any ABI detection system running POP-7, POP-37 or POP-4, the samples will have to be transferred away from the beads into a clean reaction plate prior to loading (see step 8). Do not denature the samples, because this will break down the dyes.

For ABI 3100 users please see the application note on *Agencourt CleanSEQ on the ABI 3100*

ABI Capillary Sequencers 3100, 3700 & 3730: The suggested elution buffers are 0.1mM EDTA (pH 8.0), 0.5mM EDTA (pH 8.0) or reagent grade water*. The appropriate elution buffer will vary depending on the sensitivity of the sequencing detector, the amount of BigDye used per sequencing reaction AND the type of template. For samples being inclined to have high signals, such as PCR products, reactions with more than 2µL of Big Dye Terminator and samples loaded on ABI 3730 or 3100, EDTA should be used for elution. High signals can lead to overloading and EDTA helps to even out sample injection to counteract this effect (see Figure 1 on the next page). Water elution is recommended when the user is trying to maximize the signal, for example plasmids and low copy vector sequences, reactions with less than 2µL of BigDye Terminator and samples loaded on ABI 3700. Please use table 3 as a general guideline for choosing an elution buffer. Refer to figure 2 for the effects of loading solutions.

Table 3

	ABI 3100	ABI 3700	ABI 3730
>2 µL BigDye with PCR Products	0.1mM EDTA	0.1mM EDTA	0.1mM EDTA
<2 µL BigDye with PCR Products	0.1mM EDTA	DiH_2O	0.1mM EDTA
>2 µL BigDye with Plasmids	0.1mM EDTA	DiH_2O	0.1mM EDTA
<2 µL BigDye with Plasmids	DiH_2O	DiH_2O	DiH_2O

***Note: Formamide can also be used as an elution buffer for ABI samples, though it may reduce the signal of your samples. When using formamide as an elution buffer, seal and vortex the plate for 30 seconds before loading them. Additionally, when formamide absorbs moisture from the air it creates formic acid, which can break apart some of the incorporated dyes and cause dye blobs.**

Beckman Coulter CEQ Sequencers: The suggested elution buffer is deionized formamide (SLS from Beckman or Hi-Di formamide from ABI). When using formamide as an elution buffer, seal and vortex the plate for 30 seconds before loading them. CEQ samples can actually be eluted immediately after removing the final ethanol wash, without observing the 10 minute drying time. The recovery of sequencing extension products from the magnetic beads is dependent on the quality of the deionized formamide. Formamide having been frozen and thawed multiple times may only yield partial elution of the sequencing products, leading to decreases in signal intensity/quality of your sequencing samples.

Figure 1. Example of an overloaded ABI 3700 capillary

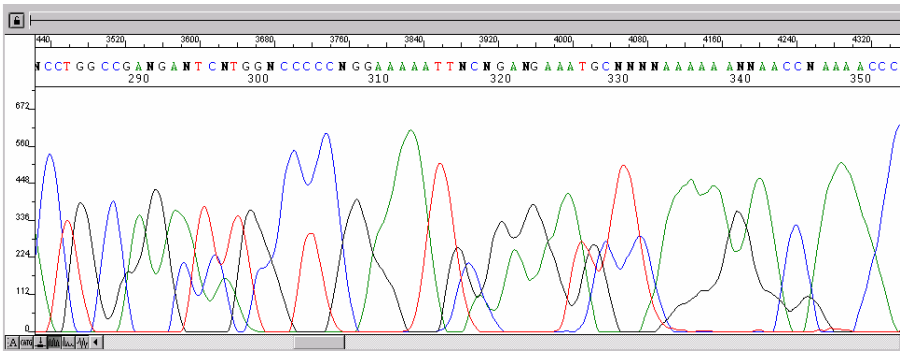
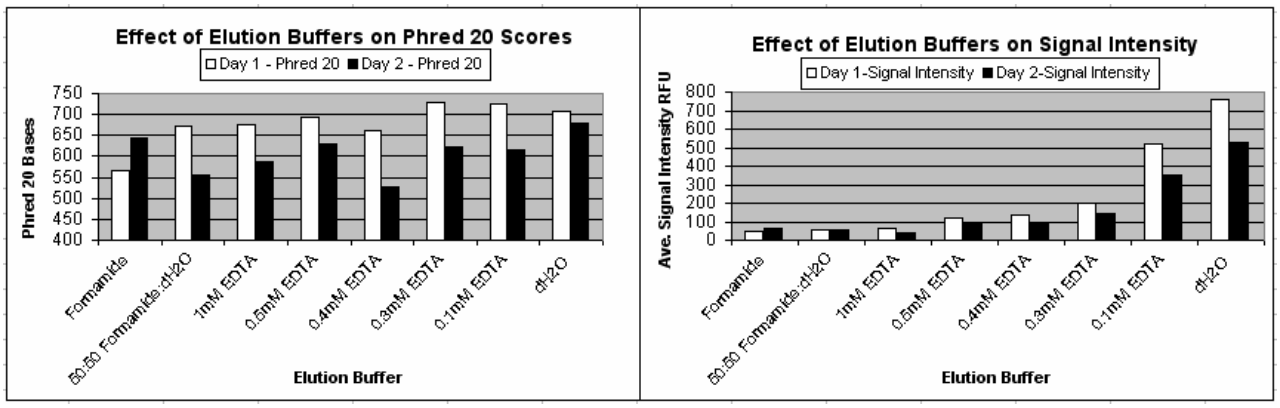


Figure 2. Phred 20 scores and signal intensity of different elution buffers



8. **This final step is only necessary for ABI detection systems running with POP 37, POP 4 and POP 7: Place the reaction plate onto an Agencourt SPRIPlate 96R to separate the beads from solution. When the separation is complete, transfer the cleared samples into a clean plate for loading on the detector.**

   The recommended separation time is 3 minutes. The solution should be clear before transferring samples to the new plate. Magnetic beads will interfere with sample injection when POP- 37, POP-7 or POP-4 is used. Therefore it is very important to remove the beads as completely as possible prior to loading. Leaving 5 µL of liquid behind in the elution plate can help to prevent transfer of beads into the loading plate.

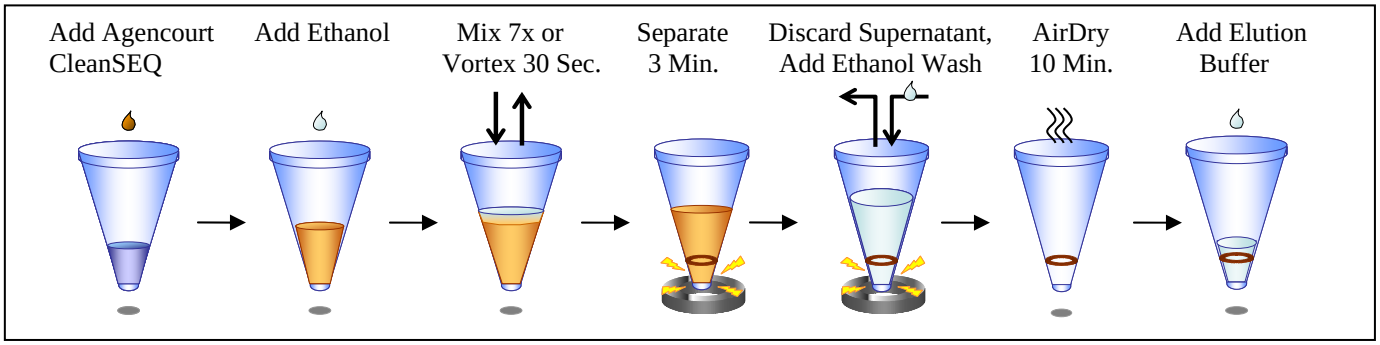
<u>Agencourt CleanSEQ Quick Reference for 96 Well Format:</u>

1. **Gently shake the Agencourt CleanSEQ bottle to resuspend any magnetic particles that may have settled. Add 10 µL of Agencourt CleanSEQ to the reaction plate.**
2. **Add ___ µL 85% ethanol to the reaction plate (according to the table in the appropriate protocol). Pipette mix 7 times, or seal and vortex the reaction plate for 30 seconds.**
3. **Place the reaction plate onto an Agencourt SPRIPlate 96R for 3 minutes to separate beads from solution.**
4. **Aspirate the cleared solution (supernatant) from the reaction plate and discard.**
5. **Dispense 100 µL of 85 % ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. Note: A second wash is required if using 4µL of BigDye or more per reaction.**
6. **Let the reaction plate air-dry for 10 minutes at room temperature.**

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

7. **Add 40 µL of elution buffer (see protocol) and incubate the plate for 5 minutes at room temperature to elute.**

8. **This final step is only NECESSARY FOR <u>ABI 3730, ABI POP 37, ABI POP 7, ABI POP 4</u> and <u>MegaBACE</u> USERS: Place the reaction plate onto an Agencourt SPRIPlate 96R for 3 minutes to separate beads from solution. When separation is complete, transfer cleared samples over into a new clean reaction plate for loading on the detector.**



ABI BigDye Terminator/Beckman Coulter CEQ DTCS 384 Well Format:

1. **Gently shake the Agencourt CleanSEQ bottle to resuspend any magnetic particles that may have settled. Add 5 µL of Agencourt CleanSEQ to the reaction plate.**

   This step should be performed **before** the plate is placed on the Agencourt SPRIPlate. Use 5 µL of Agencourt CleanSEQ regardless of the sequencing reaction volume.

2. **Add 85% ethanol to the reaction plate according to the table below. Pipette mix 7 times.**

   This step should also be performed **before** placing the plate on the Agencourt SPRIPlate. If you are vortexing, use a medium speed (6-8 on a standard mini vortexer) and make sure the suspension is completely homogeneous before continuing. The volume of 85% ethanol needed has been calculated for common sequencing reaction volumes, as shown in Table 2. To determine the volume of ethanol needed for other sequencing reaction volumes use the equation provided below or use the Agencourt Agencourt CleanSEQ calculator at http://www.agencourt.com/technical/reagent_information/cleanseq/

   Editors Note: There has been a change at this step for Agencourt CleanSEQ 384. We have concluded, after testing, that 7 tipmixes are sufficient to obtain a completely homogeneous suspension whereas the previous protocol recommended 14 tipmixes .

Table 2

Agencourt CleanSEQ 96 & 384 for ABI and CEQ Dye Terminators - Volume of ethanol (µL) to add to sequencing reactions of different volumes.

Volume of Sequencing Reaction (µL)	Volume of 85% Ethanol (96Well)	Volume of 85% Ethanol (384Well)
5	31	14.3
10	42	21.4
15	52	28.6
20	62	
25	73	

The volume of EtOH to add to each reaction can be determined by the following equation:

$$Vol_{EtOH} = [(Vol_B + Vol_S) \times (C_F/C_E)]/[1 - (C_F/C_E)]$$

Where:

Vol_{EtOH} = volume of ethanol to be used per reaction

Vol_B = volume of Agencourt CleanSEQ used, 5µL for 384 well format

Vol_S = volume of the DNA sample to be cleaned up

C_F = final concentration of EtOH, 50% for 384 well format

C_E = concentration of ethanol to be used (Agencourt recommends 85% ethanol)

Note:	**If you are using a lower concentration of ethanol, the volume of ethanol needed may exceed the maximum volume of the wells in a standard 384 well plate. If this happens, use a higher concentration of ethanol or transfer a part of the reaction into a new 384 well plate.**

3. **Place the reaction plate on an Agencourt SPRIPlate 384 for 2 minutes to separate beads from solution.**

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

4. **Aspirate the cleared solution (supernatant) from the reaction plate and discard it.**

   This step should be performed while the plate is situated on the Agencourt SPRIPlate 384. Be careful not to disturb the beads. It is important to completely remove all of the supernatant, as it contains excess fluorescent dye and contaminants.

5. **Dispense 30 µL of 85% ethanol and pipette mix 1-3 times, incubate 30 seconds at room temperature, then aspirate out the ethanol and discard. Repeat for a total of 2 washes.**

   This step should be performed while the plate is situated on an Agencourt SPRIPlate 384. The number of pipette mixes depends on the automation platform. There is no need to agitate the beads.

6. **Let the reaction plate air-dry for 10 minutes at room temperature.**

   The reaction plate does not have to be situated on the Agencourt SPRIPlate 384 during drying. Note that excessive drying can lead to degradation of incorporated dye terminators. After removing the final ethanol wash, allow the samples to dry at room temperature for approximately 10 minutes. The plate may then be sealed and stored at 4°C or -20°C. Elute the samples just prior to loading on the sequencing detector.

7. **Add 15 µL of elution buffer and incubate the plate for 5 minutes at room temperature.**

   Elution of the sequencing products from the magnetic beads is rapid and it is not necessary for the beads to go back into solution for complete recovery of the product. For ABI 3100 and 3700 running with POP-5 or POP-6 and the Beckman Coulter CEQ, eluted plates can be loaded directly onto the detector without removal of the beads. For ABI 3730 or any ABI detection system running POP-7, POP-37 or POP-4, the samples will have to be transferred

away from the beads into a clean reaction plate prior to loading (see step 8). Do not denature the samples, because this will break down the dyes.

ABI Capillary Sequencers 3100, 3700 & 3730: The suggested elution buffers are 0.1mM EDTA (pH 8.0), 0.5mM EDTA (pH 8.0) or reagent grade water*. The appropriate elution buffer will vary depending on the sensitivity of the sequencing detector, the amount of BigDye used per sequencing reaction AND the type of template. For samples being inclined to have high signals, such as PCR products, reactions with more than 2 µL of Big Dye Terminator and samples loaded on ABI 3730 or 3100, EDTA should be used for elution. High signals can lead to overloading and EDTA helps to even out sample injection to counteract this effect (Figure 1 on page 6). Water elution is recommended when the user is trying to maximize the signal, for example plasmids and low copy vector sequences, reactions with less than 2 µL of BigDye Terminator and samples loaded on ABI 3700. Please use table 3 as a general guideline for choosing an elution buffer. See Figure 2 on page 7 for the effects of loading solutions.

Table 3

	ABI 3100	**ABI 3700**	**ABI 3730**
>2 µL BigDye with PCR Products	0.1mM EDTA	0.1mM EDTA	0.1mM EDTA
<2 µL BigDye with PCR Products	0.1mM EDTA	DiH_2O	0.1mM EDTA
>2 µL BigDye with Plasmids	0.1mM EDTA	DiH_2O	0.1mM EDTA
<2 µL BigDye with Plasmids	DiH_2O	DiH_2O	DiH_2O

Note: Formamide can also be used as an elution buffer for ABI samples, though it may reduce the signal of your samples. When using formamide as an elution buffer, seal and vortex the plate for 30 seconds before loading them. Additionally, when formamide absorbs moisture from the air it creates formic acid, which can break apart some of the incorporated dyes and cause dye blobs.

Beckman Coulter CEQ Sequencers: The suggested elution buffer is deionized formamide (SLS from Beckman or Hi-Di formamide from ABI). When using formamide as an elution buffer, seal and vortex the plate for 30 seconds before loading them. CEQ samples can actually be eluted immediately after removing the final ethanol wash, without observing the 10 minute drying time. The recovery of sequencing extension products from the magnetic beads is dependent on the quality of the deionized formamide. Formamide having been frozen and thawed multiple times may only yield partial elution of the sequencing products, leading to decreases in signal intensity/quality of your sequencing samples.

8. **This final step is only necessary for ABI detection systems running with POP 37, POP 4 and POP 7: Place the reaction plate onto an Agencourt SPRIPlate 384 to separate the beads from solution. When the separation is complete, transfer the cleared samples into a clean plate for loading on the detector.**

   The recommended separation time is 3 minutes. The solution should be clear before transferring samples to the new plate. Magnetic beads will interfere with sample injection when POP- 37, POP-7 or POP-4 is used. Therefore it is very important to remove the beads as completely as possible prior to loading. Leaving 3µL of liquid behind in the elution plate can help to prevent transfer of beads into the loading plate.

Agencourt CleanSEQ for Amersham Biosciences DYEnamic™ ET Dye Terminators

The Amersham ET Terminator Agencourt CleanSEQ reaction clean-up protocol can be used for 96 and 384 well formats. The following table shows the number of reactions that each kit will yield:

Table 4

	APN 000121 (8mL)	*APN 000136 (50mL)*
96 or 384 Well Format	1600	10000

Amersham Biosciences DYEnamic™ ET Dye Terminators 96 Well Format:

1. **Gently shake the Agencourt CleanSEQ bottle to resuspend any magnetic particles that may have settled. Add 5µL of Agencourt CleanSEQ to the reaction plate.**

   This step should be performed **before** the plate is placed on the Agencourt SPRIPlate. Use 5 µL of Agencourt CleanSEQ regardless of the sequencing reaction volume.

2. **Add 85 % ethanol to the reaction plate according to the table below. Pipette mix 7 times or seal and vortex the reaction plate for 30 seconds.**

   This step should also be performed **before** placing the plate on the Agencourt SPRIPlate. If you are vortexing, use a medium speed (6-8 on a standard mini vortexer) and make sure the suspension is completely homogeneous before continuing. The volume of 85% ethanol needed has been calculated for common sequencing reaction volumes, as shown in Table 5. To determine the volume of ethanol needed for other sequencing reaction volumes use the equation provided below or use the Agencourt Agencourt CleanSEQ calculator at http://www.agencourt.com/technical/reagent_information/cleanseq/

Table 5

Agencourt CleanSEQ 96 for Amersham ET Dye Terminators *- Volume of ethanol (µL) to add to sequencing reactions of different volumes.*	
Volume of Sequencing Reaction (µL)	Volume of 85% Ethanol (96Well)
5	47
10	70
15	93
20	117

The volume of EtOH to add to each reaction can be determined by the following equation:

$$Vol_{EtOH} = [(Vol_B + Vol_S) \times (C_F/C_E)]/[1 - (C_F/C_E)]$$

Where:

Vol_{EtOH} = volume of ethanol to be used per reaction

Vol_B = volume of Agencourt CleanSEQ used (5µL)

Vol_S = volume of the DNA sample to be cleaned up

C_F = final concentration of ethanol (70%)

C_E = concentration of ethanol to be used (Agencourt recommends 85% ethanol)

3. **Incubate the reaction plate for at least 3 minutes at room temperature.**

   This incubation must be performed before the reaction plate is placed on the Agencourt SPRIPlate.

4. **Place the reaction plate onto the Agencourt SPRIPlate 96R for 3 minutes to separate beads from solution.**

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

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

This step should be performed while the plate is situated on the Agencourt SPRIPlate96-R. Be careful not to disturb the beads. It is important to completely remove all of the supernatant, as it contains excess fluorescent dye and contaminants.

6. **Dispense 100µL of 85 % ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. (If a few dye blobs are observed at the front of the sequencing trace, a second wash with 100µL of 85% may be necessary to wash out all of the unincorporated dye.)**

   This step should be performed while the plate is situated on the Agencourt SPRIPlate96-R. There is no need to agitate the beads. It is important to remove all of the ethanol, as it contains residual fluorescent dye and contaminants.

7. **Let the reaction air-dry for 10 minutes at room temperature.**

   The reaction plate does not have to be situated on an Agencourt SPRIPlate 96R during drying. Note that excessive drying can lead to degradation of incorporated dye terminators. After removing the final ethanol wash, allow the samples to dry at room temperature for approximately 10 minutes. The plate may then be sealed and stored dry at 4°C or -20°C. Elute the samples just prior to loading on the sequencing detector.

8. **Add 30 µL of elution buffer and incubate the plate for 5 minutes at room temperature to elute.**

   The elution of sequencing products from the magnetic beads is rapid, and it is not necessary for the beads to go back into solution for complete recovery of the samples. Suggested elution buffers are reagent grade water (optimal), 0.1mM EDTA (pH 8.0), or 0.5mM EDTA(pH 8.0)*. For maximum signal intensity, elute samples in reagent grade water. If overloading of sample occurs, using 0.1mM EDTA as an elution buffer can help to temper the signal and improve readlengths and Phred 20 scores. If overloads are still seen with 0.1 mM EDTA, use 0.5 mM EDTA instead. An increase in the concentration of EDTA will decrease the signal strength.

***Note:**	**Formamide can also be used as an elution buffer for ET Terminator samples, though it may reduce the signal of your samples. When using formamide as an elution buffer, seal and vortex the plates for 30 seconds before loading them. Additionally, when formamide absorbs moisture from the air, it creates formic acid, which can break apart some of the incorporated dyes and cause dye blobs.**

   This final step is only NECESSARY FOR MEGABACE™ USERS: Place the reaction plate onto an Agencourt SPRIPlate 96R to separate beads from solution. When separation is complete, transfer cleared samples into a clean plate for loading on the MegaBACE.

The recommended separation time is 3 minutes. The solution should be clear before transferring samples to the new plate. Magnetic beads will interfere with sample injection on the MegaBACE by causing fluctuations in the current during the sequencing run. It is very important to remove the beads as completely as possible prior to loading. Leaving 5µL of liquid behind in the elution plate can help to prevent transfer of beads into the loading plate. (Customers using ET Terminators on ABI capillary sequencing systems 3100 & 3700 may load sample and beads together on their capillary systems, unless the detectors are using POP 7, POP 37 or POP 4)

Agencourt CleanSEQ for Amersham Biosciences DYEnamic ET Dye Terminators 384 Well Format:

Important: For optimal mixing within the relatively small volume of a 384 well plate, it is recommended that no more than 10 µL of reaction be purified. Due to volume constraints, purification of a 10 µL reaction may not be possible in all 384 well cycle plates. In these cases, it is suggested that 5 µL of the 10 µL (or greater volume) reaction be transferred into a new 384 well plate for cleanup. This transfer may also help to normalize the cleanup if evaporation within the 384 well plate is a problem during thermal cycling.

1. **Gently shake the Agencourt CleanSEQ bottle to resuspend any magnetic particles that may have settled. Add 5 µL of Agencourt CleanSEQ to the reaction plate.**

   This step must be performed **before** the plate is placed on the Agencourt SPRIPlate. Use 5 µL of Agencourt CleanSEQ regardless of the sequencing reaction volume.

2. **Add 100% ethanol to the reaction plate according to the table below. Pipette mix 14 times.**

   This step must be performed **before** placing the plate on the Agencourt SPRIPlate. The volume of ethanol needed has been calculated for common sequencing reaction volumes, as shown in table 6. To determine the volume of ethanol needed for other sequencing reaction volumes, use the equation provided on page 16.

Table 6

Volume of Sequencing Reaction (µL)	Volume of 100% Ethanol (384-Well)
5	23
10	35

The volume of EtOH to add to each reaction can be determined by the following equation:

$$Vol_{EtOH} = [(Vol_B + Vol_S) \times (C_F/C_E)]/[1 - (C_F/C_E)]$$

Where:

Vol_{EtOH} = volume of ethanol to be used per reaction

Vol_B = volume of Agencourt CleanSEQ used (5µL)

Vol_S = volume of the DNA sample to be cleaned up

C_F = final concentration EtOH (70%)

C_E = concentration of ethanol to be used (Agencourt recommends 100% ethanol)

3. **Incubate the reaction plate for at least 3 minutes at room temperature.**

   This incubation must be performed before the reaction plate is placed on the Agencourt SPRIPlate.

4. **Place the reaction plate on the Agencourt SPRIPlate 384 for two minutes to separate the beads from solution.**

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

5. **Aspirate cleared solution (supernatant) from the reaction plate and discard it.**

   This step should be performed while the reaction plate is situated on the Agencourt SPRIPlate 384. Be careful not to disturb the beads. It is important to completely remove all of the supernatant as it contains excess fluorescent dye and contaminants.

6. **Dispense 40 µL of 85% ethanol and pipette mix 1-3 times, incubate 30 seconds at room temperature, then aspirate out the ethanol and discard. Repeat for a total of 2 washes.**

   This step should be performed carefully while the plate is situated on an Agencourt SPRIPlate 384. There is no need to agitate the beads.

7. **Let the reaction plate air-dry for 10 minutes at room temperature.**

   The reaction plate does not have to be situated on an Agencourt SPRIPlate 384 during drying. Note that excessive drying can lead to degradation of incorporated dye terminators. After removing the final ethanol wash, allow the samples to dry at room temperature for approximately 10 minutes. The plate may then be sealed and stored at 4°C or -20°C. Elute the samples just prior to loading on the sequencing detector.

8. Add 15 µL of elution buffer and incubate the plate for 5 minutes at room temperature.

Elution of sequencing products from the magnetic beads is rapid and it is not necessary for the beads to go back into solution for complete recovery of the product. Suggested elution buffers are reagent grade water (optimal), 0.1mM EDTA (pH8.0), or 0.5 mM EDTA (pH8.0)*. For maximum signal intensity, elute samples in reagent grade water. If overloading of sample occurs, using 0.1mM EDTA as an elution buffer can help to temper the signal and improve readlengths and Phred 20 scores. If overloads are still seen with 0.1 mM EDTA, use 0.5 mM EDTA instead. An increase in the concentration of EDTA will decrease the signal strength.

***Note: Formamide can also be used as an elution buffer for ET Terminator samples, though it may reduce the signal of your samples. When using formamide as an elution buffer, seal and vortex the plates for 30 seconds before loading them. Additionally, when formamide absorbs moisture from the air, it creates formic acid, which can break apart some of the incorporated dyes and cause dye blobs.**

This final step is only NECESSARY FOR MEGABACE USERS: Place the reaction plate onto the Agencourt SPRIPlate 384 to separate the beads from solution. When the separation is complete, transfer the cleared samples into a clean reaction plate for loading on the MegaBACE.

The recommended separation time is 3 minutes. The solution should be clear before transferring samples to the new plate. Magnetic beads will interfere with sample injection on the MegaBACE by causing fluctuations in the current during the sequencing run. It is very important to remove the beads as completely as possible prior to loading. Leaving 3 µL of liquid behind in the elution plate can help to prevent transfer of beads into the loading plate. (Customers using ET Terminators on ABI capillary sequencing systems 3100 & 3700 may load sample and beads together on their capillary systems, unless the detectors are using POP 7, POP 37 or POP 4)

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