Ethanol/EDTA/Sodium Acetate Precipitation For Big Dye v. 3.1 Chemistry

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SUMMARY

This protocol outlines the procedure of cleaning up sequencing reactions with Big Dye v. 3.1 Chemistry.

MATERIALS & REAGENTS

Materials/Reagents/Equipment	*Vendor*	*Catalog Number*
DISPOSABLES		
Half Skirted 96 well Plates	ISC BioExpress	T-3060-1
Stack Rack 1uL-200 uL Natural Tip	USA Scientific	1111-0200
TempPlate Sealing Film	USA Scientifc	2921-0000
Blue Paper Towels	Vet Med	
REUSABLES		
Polymer PCR Sealing Mats	ISC BioExpress	T-3161-1
Foil Stripper	Orochem Technologies	OT-0592
REAGENTS		
EtOH/EDTA/NaOAc	Homemade	
70% Ethanol	Homemade	
3M NaOAc	Tek Nova	S0296
95% Ethanol	Vet Med	
100% Ethanol	Vet Med	
DDH20	Millipore System	
125mM EDTA	Homemade	
EQUIPMENT		
Centrifuge	Beckman Coulter	
SpeedVac	Savant	

PROCEDURE

1. Make sure the centrifuge is turned on and set at 4 °C . Sign up for the SpeedVac for 30 minutes.
2. Take out the sequencing plates from Freezer C. Use the foil stripper to pull off the seal.
3. For a 5uL reaction volume, add 35 uL EtOH/EDTA/NaOAc (stored at 4 °C) mix to each well.
4. Seal the plate well with a polymer PCR sealing mat using a roller. Take a pipette tip box and hold the plate against it tightly and mix by inverting it at least 4 times or vortexing it for 15 seconds. Note: Make sure there is no leakage as this may contaminate the samples.
5. Number each plate from 1-8.
6. Leave the reaction plate at room temperature for at least 15 minutes to precipitate the extension products.
7. Place the samples in the centrifuge set at 4 °C for 30 minutes at 3250 RPM. Note: Always place a purple/pinkish pipette tip rack under the 96 well plates when spinning. This will ensure that the wells of the plate will not break off.
8. While the samples are spinning for 30 minutes, fold as many paper towels needed into thirds.
9. Remove the samples from the centrifuge **IMMEDIATELY** and place the folded paper towels on the centrifuge racks. Remove the mat and place the plate face down on a paper towel in the centrifuge rack. NOTE: place the PCR seal mats corresponding to the number on the plate. This will help you use the same mat for the plate in the following steps. Set the centrifuge at 750 RPM and spin up to 750 RPM, then remove from the centrifuge. Once stopped, place the plates upright on the benchtop and discard the folded paper towels. Repeat this with the other samples, as only four can be spun at a time.
   IMPORTANT! The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the wells, the more unincorporated dye terminators will remain in the samples.
10. Rinse samples by adding 35 uL of 70% EtOH (stored at –20 C in Freezer C) to each pellet.
11. Seal the plate well the same PCR seal mats used in the above steps, then invert the reaction plate a few times or vortex for 15 seconds to mix.
12. Place the samples in the centrifuge at 2600 RPM for 15 minutes.
13. Decant the liquid using the same procedure in STEP 9. Note: Another 70% EtOH can be done at this point, but is not necessary unless dye blobs are seen during the sequencing run.
14. Place the plates in the SpeedVac for 15 minutes on Medium heat. If the SpeedVac is not available then place the plates in the hood for 30 minutes with the blower turned on.
15. Seal the plates with ThermalSeal Tape and place them in Freezer C. Now they are ready to be ran on the sequencer.

Reagent Recipes

EtoH/EDTA/NaOAc Mix for 108 mL
87 mL 95% EtOH
3 mL 3M NaOAc
3 mL 125mM EDTA
15 mL ddH20 HPLC Grade
Mix well and store in 4 °C .

70% EtOH for 5 L
3500 mL 100% EtOH
1500 mL ddH20
Mix well and store in –20 °C Freezer C.
NOTE: 70% ethanol does not freeze so if it is frozen, you made it wrong.

0.5 M EDTA 1L
186.1 g Disodium EDTA 2H20
Add this to 800 mL of water and stir vigorously. Adjust the ph 8.0 with NaOH and Autoclave.

Dilution from 0.5 M EDTA(100 mL) to 125 mM EDTA for 500 mL

Add 400 mL of HPLC grade H20
Add 100 mL of 0.5 M EDTA