Pellet Paint® NF Co-Precipitant

About the Kits

Pellet Paint NF Co-Precipitant	125 rxn	70748-3
	1000 rxn	70748-4

Description

Pellet Paint NF Co-Precipitant is a non-fluorescent, dye-labeled carrier compatible with fluorescent sequencing. It facilitates rapid removal of BigDye® Terminators during the alcohol precipitation of cycle sequencing reaction products. Cycle sequencing reactions can be precipitated rapidly with centrifugation times of 10 minutes. The easily visualized carrier provides a simple confirmation that precipitation has occurred. Sequencing reaction products are efficiently pelleted and dye-labeled terminators remain in the supernatant during alcohol precipitation using the standard Applied Biosystems precipitation protocol. Resuspension of pelleted sequencing reaction products in deionized formamide can be confirmed by checking for dissolution of the carrier pellet. Pellet Paint NF Co-Precipitant is fully compatible with the ABI PRISM® BigDye Terminator Cycle Sequencing Ready Reaction. To avoid extra sample handling, Pellet Paint NF Co-Precipitant can be added directly to the reaction mix, template DNA, crude PCR samples, or dilution buffer prior to the cycle sequencing reaction.

The use of Pellet Paint NF Co-Precipitant does not require the use any salts beyond those already present in the standard BigDye Ready Reaction Mix. Sodium Acetate is included for other precipitation applications where the addition of salt may be required.

Although Pellet Paint NF Co-Precipitant absorbs in the UV range, accurate spectrophotometric measurements of DNA or RNA samples are possible; the absorbance ratio, which is provided with each package of Pellet Paint NF Co-Precipitant, can be used as a correction factor when determining nucleic acid concentration. Pellet Paint NF Co-Precipitant has no detectable effect on the sequencing reaction or sequence accuracy. Pellet Paint NF Co-Precipitant is a useful substitute for the original Pellet Paint in applications where fluorescent detection is used.

Components

- 125 µl or 1 ml — Pellet Paint NF Co-Precipitant
- 1 or 8 ml — 3M Na Acetate, pH 5.2

Storage

Store at –20°C.

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Cycle Sequencing with Pellet Paint® NF Co-Precipitant

Pellet Paint NF Co-Precipitant can be included during the assembly of cycle sequencing reactions without affecting the sequencing reaction. No extra pipetting steps are required for incorporation of the carrier. Conveniently, Pellet Paint NF Co-Precipitant can be added to individual reaction components, such as template, primer, or water, such that the same amount of Pellet Paint NF Co-Precipitant is delivered to a sequencing reaction each time the component is used. The presence of the carrier has no effect on the performance of BigDye® cycle sequencing reactions.

Whether performing 10 or 20 µl reactions, use 1 µl of Pellet Paint NF Co-Precipitant to assure pellet visibility. Do not exceed 1 µl/reaction for sequencing applications, as the pellet mass can cause the pellet to dislodge during the inverted supernatant removal spins. Follow the recommendations for amounts of template, primer, and thermal cycling conditions, as listed in the ABI BigDye cycle sequencing protocol (3). An example of a sequencing reaction incorporating Pellet Paint NF Co-Precipitant is described below. Thaw Pellet Paint NF Co-Precipitant completely prior to use.

Example sequencing reaction

Amount	Component
8 µl	Terminator Ready Reaction Mix
50–100 ng	Template ssDNA
200–500 ng	dsDNA
30–90 ng	PCR DNA
3.2 pMol	Primer
1 µl	Pellet Paint NF Co-Precipitant
to 20 µl	deionized water

Vortex mixture gently. Centrifuge briefly. Cycle according to conditions described in ABI protocol.

Note: *Exceeding 1µl/reaction of Pellet Paint NF Co-Precipitant with MicroAmp® tubes may increase the risk that precipitated material will dislodge during the inverted spin.*

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Precipitation of Cycle Sequencing Reactions

The sequencing reaction products can be separated from unincorporated nucleotides and BigDye® terminators by precipitation with alcohol, as described in the ABI protocols. The use of Pellet Paint® NF Co-Precipitant will improve the efficiency and reliability of the precipitation, and reduce the time required for precipitation and centrifugation steps. When reactions precipitated with Pellet Paint NF Co-Precipitant were compared to controls lacking carrier, the reaction signal strength observed was up to five-fold higher

When using MicroAmp® trays and tray centrifugation, the ethanol precipitation protocol (without additional sodium acetate) is recommended. This method produces the firmest, most adherent pellets, reducing the risk of pellet loss. Any of the ABI precipitation protocols are suitable for microcentrifuge applications.

The table below presents a summary of the precipitation procedures for cycle sequencing reactions. Except for the reduction in centrifugation times, the protocols are nearly identical to those described in the ABI BigDye cycle sequencing manual. Perform all steps at room temperature.

Guidelines for precipitation of cycle sequencing reactions

Sample	BigDye Cycle Sequencing Reactions, 20 µl reaction Volume			
Tube type	MicroAmp tray		0.5 ml PCR Tube	
Alcohol	**Ethanol, 95%**	Isopropanol	**Ethanol, 95%**	Isopropanol
Add water	**16 µl**	20 µl	**16 µl**	20 µl
Add alcohol	**64 µl**	60 µl	**64 µl**	60 µl
% Alcohol, final	**60% (+/–3%)**	60% (+/–5%)	**60% (+/–3%)**	60% (+/–5%)
Centrifugation	**3000 × g, 10 min**		**≥ 10,000 × g, 3 min**	
Decant, add 70% ethanol wash	**not required for MicroAmp trays**		**250 µl**	250 µl
Repeat spin			**≥ 10,000 × g, 3 min, dry pellet, 90°C**	
Inverted tray spin	**≤ 500 × g, 1 min**		**not required for PCR tubes**	
Resuspension	Resuspend pellets in TSR at volumes recommended in the ABI manual			

Notes: *Ethanol precipitation (bold) is recommended. Do not use isopropanol for washing the pellet. Prepare an alcohol and water stock to reduce the number of pipetting steps. For example, a stock of 75% ethanol can be prepared in sterile water. To each 20 µl cycle sequencing reaction, add 80 µl 75% ethanol to give a final concentration of 60% ethanol.*

Use the lowest speed possible during the inverted spin steps. Monitor the paper towel for evidence that the pellet has dislodged. The centrifugation steps may require optimization for your particular tube/centrifuge combination. If the pellets dislodge, increase the time of the centrifugation step, and reduce the speed and duration of the inverted spin. If the problem persists, decrease the amount of Pellet Paint NF Co-Precipitant to 0.5 µl/reaction.

Precipitation of PCR reactions for cycle sequencing

PCR reaction products can be precipitated in the presence of Pellet Paint® NF Co-Precipitant, allowing the removal of excess primers and dNTPs. This protocol does not require the addition of salt prior to precipitation.

Note: This protocol uses 2 µl Pellet Paint NF Co-Precipitant for a 50 µl PCR reaction; thus, if the equivalent of 3 µl of the original 50 µl PCR volume is used for sequencing, the carryover of Pellet Paint NF Co-Precipitant in the cycle sequencing reaction will be negligible (0.12 µl). However, using a larger proportion of the precipitated PCR reaction in a cycle sequencing reaction will carry over more Pellet Paint NF Co-Precipitant. To avoid exceeding the recommended limit of 1 µl Pellet Paint NF Co-Precipitant per sequencing reaction, decrease the amount of Pellet Paint NF Co-Precipitant added to the sequencing reaction mix.

Ethanol precipitation of PCR reactions

1. When performing PCR reactions with oil overlay, carefully remove lower aqueous phase and transfer to clean 1.5-ml microcentrifuge tube.
2. For each 25 µl PCR reaction volume, add 1 µl Pellet Paint NF Co-Precipitant.
3. To the total volume, add 1 vol ethanol.
4. Vortex gently and incubate at room temperature for 1 min.
5. Spin at top speed in a microcentrifuge for 5 min.
6. A dark blue pellet should be visible at the bottom of the tube. Remove as much supernatant as possible without disturbing pellet.
7. Wash pellet with 500 µl 70% ethanol. Vortex briefly. Centrifuge for 3 min.
8. Carefully remove all of the supernatant, without disturbing pellet.
9. Dry pellet in vacuum centrifuge, or at 90°C on a heat block.
10. Resuspend pellet in appropriate volume of deionized water.

General Precipitation of Nucleic Acids with Pellet Paint NF Co-Precipitant

Pellet Paint NF Co-Precipitant can be used in a manner similar to the original Pellet Paint for the precipitation of nucleic acids. Note that Pellet Paint NF Co-Precipitant has only been qualified for precipitation of PCR products and cycle sequencing. Effects of Pellet Paint NF Co-Precipitant use in other applications must be determined empirically. This protocol requires the use of sodium acetate.

1. Thaw Pellet Paint NF Co-Precipitant and 3 M Na Acetate, pH 5.2, and bring to room temperature.
2. Add 2 µl Pellet Paint NF Co-Precipitant to sample, followed by 0.1 vol 3 M Na Acetate, pH 5.2.
3. Add 2 vol ethanol or 1 vol isopropanol. Vortex briefly. Incubate at room temperature for 2 min.
4. Spin sample in microcentrifuge at top speed for 5 min.
5. A dark blue pellet should be visible at bottom of tube. Remove as much of the supernatant as possible, without disturbing pellet.
6. Wash pellet with 70% ethanol. Vortex briefly. Spin, as in Step 4.
7. Wash pellet with 100% ethanol. Vortex briefly. Spin, as in Step 4.
8. Remove supernatant. Dry pellet in vacuum centrifuge or at 90°C on a heat block.
9. Resuspend pellet in deionized water or buffer, as desired.

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Spectrophotometric Measurement of Nucleic Acids

The absorbance contribution of Pellet Paint® NF Co-Precipitant in a given sample can be easily calculated. Because the absorbance ratio at 260 nm and 600 nm (A_{260}/A_{600}) is constant for each lot of Pellet Paint NF Co-Precipitant, one can calculate the Pellet Paint NF Co-Precipitant contribution to the total absorbance at 260 nm by taking an additional reading of the precipitated sample at 600 nm. Subtract the Pellet Paint NF Co-Precipitant component from the measured absorbance at 260 nm. The difference is the component contributed by the nucleic acid present in the sample.

For example, if the measured absorbance for your Pellet Paint NF Co-Precipitant-precipitated sample at 260 nm = 0.103, at 600 nm = 0.035, and the absorbance ratio provided by Novagen for this specific lot of Pellet Paint is 0.650, then the Pellet Paint NF Co-Precipitant component of the absorbance at 260 nm can be determined as follows:

Sample A_{600} × (Pellet Paint NF Co-Precipitant absorbance ratio) = Pellet Paint NF Co-Precipitant component of Sample A_{260}, where the Pellet Paint NF Co-Precipitant absorbance ratio = A_{260}/A_{600}

$$0.035 \times (0.650) = 0.023$$

To calculate the nucleic acid absorbance component, subtract the Pellet Paint NF Co-Precipitant component from the total absorbance at 260 nm.

$$0.103 - 0.023 = 0.080$$

Note: *Novagen provides the absorbance ratio with each lot of Pellet Paint NF Co-Precipitant. However, because of the variability in readings between different spectrophotometers, the highest degree of accuracy is achieved by measuring the Pellet Paint NF Co-Precipitant A_{260}/A_{600} ratio with the same instrument and solvents that you use for your nucleic acid determinations.*

References

1. McCormick, M. (1995) *in**Nova**tions*, **4a**, 10–11.
2. McCormick, M. (1996) *in**Nova**tions*, **5**, 10.
3. ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit Protocol (1998) The Perkin-Elmer Corporation, Foster City, CA.