

Pellet Paint[®] Co-Precipitant

Description

Pellet Paint Co-Precipitant	125 rxn	69049-3
	1000 rxn	69049-4

Pellet Paint* is a visible/fluorescent dye-labeled carrier formulated specifically for use in alcohol precipitation of nucleic acids (1, 2). The two-minute precipitation requires no low temperature incubation or prolonged centrifugation. Both RNA and DNA are efficiently precipitated from even very dilute solutions (2 ng/ml) and the pellet is easily located by its vivid pink color. The pellet can easily be followed during washing steps, preventing losses during handling. Pellet Paint does not interfere with many molecular biology procedures including PCR (including AMPLICOR[®] PCR testing (3), sequencing, ligation, transformation of *E. coli*, transcription, translation, cDNA synthesis, RNase protection assays (4), or restriction digestion (see Table 1, p. 2). The preparation is free of contaminating nucleic acids and nucleolytic enzymes. The 250 µl vial is sufficient reagent for 125 standard precipitations.

Although Pellet Paint is a colored molecule, nucleic acid concentrations can be determined by UV absorbance using the ratio listed on the tube label, as described in *Spectrophotometric Measurement of Nucleic Acids* on p. 4.

Pellet Paint exhibits an excitation/emission maxima of 552/570 nm. The fluorophore in Pellet Paint interferes with the rhodamine-labeled primers and terminators used by Applied Biosystems automated sequencers, and may also interfere with other methods based on rhodamine detection. Pellet Paint NF Co-Precipitant is non-fluorescent and is compatible with ABI PRISM[®] BigDye[™] Terminator Cycle Sequencing.

* Patent pending

© 2003 EMD Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany. All rights reserved. Pellet Paint, the Novagen logo and name are trademarks of EMD Biosciences, Inc. AMPLICOR is a trademark of Hoffman-La Roche, Ltd. ABI PRISM and BigDye are trademarks of Perkin-Elmer Corporation.

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

	Pellet Paint®	glycogen	tRNA
easily visualized	+	–	–
free of DNA and RNA	+	?	–
<i>compatible with:</i>			
gel electrophoresis	+	+	–
PCR amplification	+	?	–
DNA sequencing	+	+	–
restriction digestion	+	+	+
ligation	+	+	?
transformation	+	?	–
cDNA synthesis	+	?	–
kinase reactions	+	+	–
random priming	+	?	–
<i>in vitro</i> transcription	+	+	?
<i>in vitro</i> translation	+	+	+
RNase protection assay	+	?	+
phenol extraction	+	+	+
LiCl precipitation	+	+	–
bacterial electroporation	+	?	?

Table 1. Comparison of different carriers for precipitation of nucleic acids

Components

- 250 µl or 2 ml Pellet Paint® Co-Precipitant
- 1or 8 ml 3 M Na Acetate, pH 5.2

Storage

For short-term storage (less than 1 month), Pellet Paint may be stored at 4°C. For long-term storage, store at –20°C. Dispense into aliquots and keep a working aliquot at 4°C to avoid frequent freeze-thaw cycles. Pellet Paint should be stored in the dark. The 3 M Na Acetate should be stored at 4°C.

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

Precipitation Protocol

1. Bring Pellet Paint® Co-Precipitant and 3 M Na Acetate stocks to room temperature. Invert Pellet Paint stock several times until a uniform suspension is achieved. Vigorous vortexing of Pellet Paint stock is not recommended. If a precipitate is observed, gently heat Pellet Paint.
2. Add 2 µl Pellet Paint to nucleic acid sample followed by 0.1 volume 3 M Na Acetate. Mix sample briefly.

Note: 2 µl Pellet Paint should be used regardless of sample volume.

3. Add 2 vol ethanol or 1 vol isopropanol and vortex briefly. Incubate at room temperature for 2 min.
4. Spin sample in microcentrifuge at 14,000–16,000 × g for 5 min. A pink pellet should be visible at the bottom of tube.
5. Remove supernatant with a pipet tip. Use caution when removing the supernatant from isopropanol precipitations. Removal of supernatant by pouring from an inverted tube is not recommended.
6. Rinse pellet with several volumes of 70% ethanol, vortex briefly and spin again, as in Step 4 above.
7. Remove supernatant and rinse pellet in 100% ethanol. Spin again, as in Step 4 above, and remove final supernatant. Residual ethanol can be removed by air or vacuum drying.
8. Resuspend pink pellet in deionized water, or buffer of choice. Nucleic acids are fully resuspended when the Pellet Paint is in solution.

Notes:

- a. **Choice of alcohol:** Pellet Paint is compatible with both ethanol and isopropanol precipitations, although ethanol is recommended for routine use. The precipitated pellet is firmer and adheres more strongly to the tube wall with ethanol than isopropanol. Ethanol is more volatile, which facilitates removal and less salt will co-precipitate with ethanol than with isopropanol. In situations where total sample volume must be limited, isopropanol can be used without any effect on sample recovery. However, it is recommended that the pelleted material be rinsed with 70% ethanol prior to additional handling to secure the pellet to the tube wall and remove residual salt. Gel analysis of radiolabeled nucleic acids precipitated with Pellet Paint and either ethanol or isopropanol appear identical, and contain no detectable unincorporated mononucleotides.
- b. **Choice of salt:** Both sodium chloride and sodium acetate have been used in Pellet Paint precipitations with identical recovery efficiencies. However, sodium acetate is recommended for routine use due to its compatibility with downstream applications in which chloride ions may be inhibitory. Regardless of the salt chosen, pelleted material should be rinsed with ~0.5 ml 70% ethanol and dried briefly to remove residual salts. If salt carry-over is a concern, ammonium acetate is a suitable since it can be readily removed by thorough rinsing with 70% ethanol and a final drying step under vacuum.
- c. **Molecular weight cutoff:** The molecular weight cutoff for recovered precipitates using Pellet Paint is approximately 50 bases. Nucleic acids shorter than 50 bases will be recovered with decreasing efficiency and mononucleotides are not recovered. For applications such as PCR where primer removal may be desired, several serial precipitations may be required to eliminate primers. Multiple precipitations require only a single addition of Pellet Paint since it is recovered following each step. Users should carefully confirm primer removal for critical applications, particularly when the primer length approaches 50 bases.
- d. **Pellet Identification:** Pellet Paint contains a dye, which allows easy identification of the pellet under visible light when using the recommended quantity. If lower amounts of Pellet Paint are used, pellets can be identified by fluorescent excitation of the dye with long wavelength UV light (366 nm). Prolonged exposure to UV is not recommended as this can

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

bleach the dye and damage nucleic acids; brief exposure, sufficient to visualize small pellets, is acceptable. Hand-held UV light sources are recommended over transilluminators, as the total light output is lower, and wavelength is generally longer, reducing the likelihood of damage to the sample.

Spectrophotometric Measurement of Nucleic Acids

The absorbance contribution of Pellet Paint® Co-Precipitant in a given sample can be calculated easily. Because the absorbance ratio at 260 nm and 555 nm (A_{260}/A_{555}) is constant for each lot of Pellet Paint, you can calculate the Pellet Paint contribution to the total absorbance at 260 nm by taking an additional reading of your precipitated sample at 555 nm. Subtract the Pellet Paint component from the measured absorbance at 260 nm and the difference is the component contributed by the nucleic acid present in the sample.

For example, if the measured absorbance for your Pellet Paint-precipitated sample at 260 nm = 0.103, at 555 nm = 0.035, and the absorbance ratio provided by Novagen for this specific lot of Pellet Paint is 0.650, then the Pellet Paint component of the absorbance at 260 nm can be determined as follows:

Sample $A_{555} \times$ (Pellet Paint absorbance ratio) = Pellet Paint component of Sample A_{260} , where the Pellet Paint absorbance ratio = A_{260}/A_{555} .

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

To calculate the nucleic acid absorbance component, subtract the Pellet Paint 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. However, because of the variability in readings between different spectrophotometers, the highest degree of accuracy is achieved by measuring the Pellet Paint A_{260}/A_{555} ratio with the same instrument and solvents that you use for your nucleic acid determinations.

This method can be used for DNA and RNA solutions as dilute as 0.5 µg/ml and is compatible with either water or TE buffer as the solvent.

References

1. McCormick, M. (1996) *inNovations* **4a**, 10–11.
2. McCormick, M. (1996) *inNovations* **5**, 10.
3. Taggart, E.W., Byington, C.L., Hillyard, D.R., Robison, J.E. and Carroll, K.C. (1998) *J. Clin. Microbiol.* **36**, 3408–3409.
4. Scolnick, J.a.S., M.S>. (1996) *inNovations* **5**, 11.

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.