Technical Manual

Diamond™ Nucleic Acid Dye

INSTRUCTIONS FOR USE OF PRODUCT H1181.

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Diamond™ Nucleic Acid Dye

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1. Description

Diamond™ Nucleic Acid Dye[a] is a sensitive fluorescent dye that binds to single-stranded DNA, double-stranded DNA and RNA and can be used to stain and visualize nucleic acids in gels. The dye is compatible with denaturing and native agarose and polyacrylamide gels and can be imaged with any standard imaging system, such as by UV transillumination with a Polaroid or digital camera, GE ImageQuant™ or Bio-Rad Gel Doc™ systems. The concentrated dye is stable for up to 90 days at room temperature. Diamond™ Nucleic Acid Dye does not require prewashing or destaining of gels.

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
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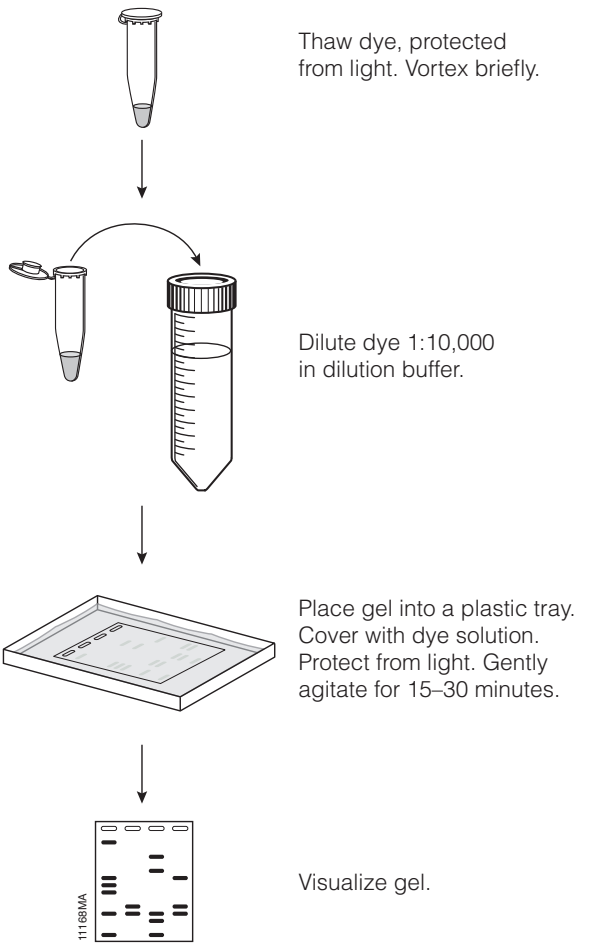

Figure 1. An overview of the Diamond™ Nucleic Acid Dye protocol.

2. Product Components and Storage Conditions

Product	Concentration	Size	Cat.#
Diamond™ Nucleic Acid Dye	10,000X in DMSO	500µl	H1181

For Research Use Only. Not for use in diagnostic procedures.

Storage Conditions: Store 10,000X dye at room temperature (22°–25°C) for up to 90 days or at –20°C for long-term storage. Protect the dye from light at all times. It is best to dilute dye immediately prior to use; however, 1X dye can be used to stain 3 gels or can be stored at room temperature in a plastic container protected from light for 3 days.

3. General Considerations

1. Diamond™ Nucleic Acid Dye has an excitation/emission profile that is similar to SYBR® Gold (494nm/558nm with double-stranded DNA) and can be imaged and documented with any system that has a SYBR® Gold filter or setting.
2. We do not recommend precasting gels with Diamond™ Nucleic Acid Dye as dye bound to DNA has been shown to interfere with electrophoretic mobility.
3. Following staining, DNA can be extracted from the gel and cleaned up using either a column-based system such as the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or ethanol precipitation.

4. Protocol for Use of Diamond™ Nucleic Acid Dye

Materials to Be Supplied By the User

- plastic staining trays
- dilution buffer (TE, TAE or TBE; see Section 4.D., Composition of Standard Gel Buffers)

4.A. Preparation of 1X Staining Solution

1. Thaw the Diamond™ Nucleic Acid Dye completely at room temperature (22°–25°C) protected from light. Vortex briefly.
2. Prepare a 1:10,000 dilution of the dye in 1X TE, TBE or TAE buffer.

   Note: For best results, the buffer used to dilute the dye should be the same as the buffer used to cast the gel and should have a pH of 7.0–8.5. Do not use water to dilute the dye. Although freshly prepared 1X dye will give best results, 1X dye can be used to stain up to 3 gels and may be stored at room temperature, in a plastic container protected from light, for 3 days.

4.B. Staining the Gel

1. Following electrophoresis, place the gel in a plastic staining tray, and completely cover the gel with staining solution.

   Note: Pipette tip box lids or similar-sized plastic containers make convenient staining trays. We do not recommend using glass containers for staining, as the dye molecules may adhere to glass surfaces.

2. Incubate the gel in staining solution at room temperature (22°–25°C) on a rocker or orbital shaker with gentle agitation, protected from light for 15–30 minutes. The time required for staining depends on the size, thickness and percentage of agarose or polyacrylamide in the gel.

4.C. Visualizing and Documenting the Gel

1. The gel may be visualized using a UV epi-illuminator or transilluminator or any other gel documentation system using a maximum excitation of 495nm.
2. The gel may be documented using Polaroid 667 film or a digital camera in combination with a 500nm cutoff filter, or another gel documentation system that can detect emission at 558nm.

4.D. Composition of Standard Gel Buffers

10X TE buffer

100mM Tris (pH 7.0–8.5)
10mM EDTA

10X TAE buffer

400mM Tris (pH 7.0–8.5)
20mM sodium acetate
10mM EDTA

10X TBE buffer

890mM Tris-base (pH 7.0–8.5)
890mM boric acid
20mM EDTA

5. Results

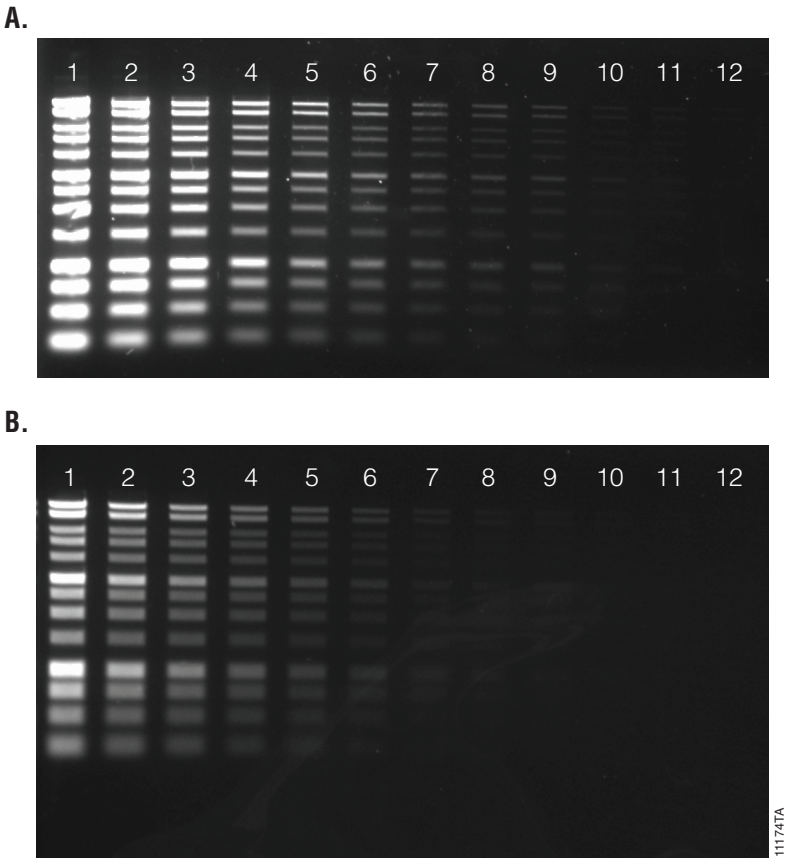

Figure 2. Staining comparison following DNA separation on a 1% agarose gel prepared with 1X TAE buffer. Ten microliters of BenchTop 1kb DNA Ladder (Cat.# G7541) was loaded into lane 1. Twofold serial dilutions of the ladder prepared in 1X Blue/Orange loading dye (Cat.# G1881) were made for lanes 2–12. **Panel A.** Following electrophoresis, the gel was incubated with 1X Diamond™ Nucleic Acid Dye for 20 minutes and imaged using the Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio-Rad) using the SYBR Gold application. **Panel B.** Following electrophoresis, the gel was stained with ethidium bromide for 20 minutes, then destained for 15 minutes and imaged using the Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio-Rad) and the ethidium bromide application.

5. Results (continued)

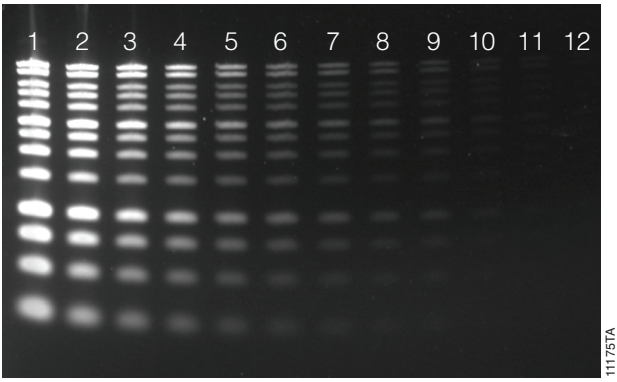

Figure 3. Diamond™ Gel Nucleic Acid staining of DNA separated on a 1.2% Clear E-gel®. Ten microliters of BenchTop 1kb DNA Ladder (Cat.# G7541) was loaded into lane 1. Twofold serial dilutions of the ladder were prepared in 1X Blue/Orange Loading Dye (Cat.# G1881) and loaded into lanes 2–12. Following electrophoresis, the gel was incubated in 1X Diamond™ Nucleic Acid Dye for 20 minutes and imaged using Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio-Rad) using the SYBR® Gold application.

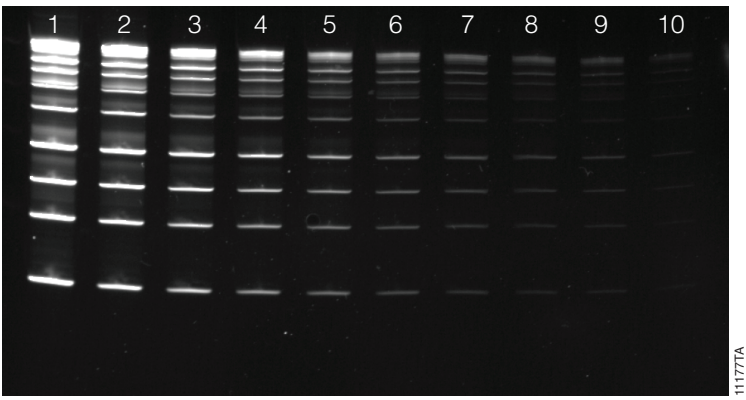

Figure 4. Diamond™ Gel Nucleic Acid staining of DNA separated on a 4–20% polyacrylamide gel. Ten microliters of BenchTop 1kb DNA Ladder (Cat.# G7541) was loaded into lane 1. Twofold serial dilutions of the ladder were prepared in 1X Blue/Orange Loading Dye (Cat.# G1881) and loaded into lanes 2–10. Following electrophoresis, the gel was incubated in 1X Diamond™ Nucleic Acid Dye for 20 minutes and imaged using Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio-Rad) using the SYBR® Gold application.

[a]Patent Pending.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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