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Novel Green Plus

Catalog Number	Unit Size	Reactions
LD003-0500	500 µl (20,000X in DMSO)	1:20,000 dilution in TE, TAE or TBE buffer

Storage : Stable for up to 1 year at -20°C.
Shipping Temperature: Room temperature

Description

The Novel Green Plus provides an easy 2-step method to stain the DNA band from DNA electrophoresis. This unique reagent ensures the DNA to be stained with a high sensitivity and good quality on the gel. Novel Green Plus is a next-generation DNA-binding dye with features ideal for use in quantitative real-time PCR (qPCR) and many other applications. We designed the dye by taking into consideration several essential dye properties relevant to PCR, including PCR inhibition, safety, and stability and fluorescence spectra of the dye. Ethidium bromide (EtBr), which presents sensitivity for detecting 1-5 ng double-stranded DNA (dsDNA) in the agarose gel analysis, has been the most common dye used for nucleic acid gel staining.

However, several drawbacks of EtBr have been understood, including that EtBr is a mutagen/carcinogen and presents a high risk of inducing cancer. Moreover, the ultraviolet (UV) light used to illuminate EtBr-DNA compounds probably results in skin or eye damage to the user if misconducted. It' s also noted that exposure to the UV light might cause chemical modifications of the DNA samples in the gel, such as the formation of TT dimers, leading to challenges with the subsequent DNA manipulations.

Reduced efficiency of transformation is observed by our scientists, after conducting ligation with the DNA samples isolated from the gel exposed to a longer period of UV illumination. As compared with EtBr, the Novel Green Plus shows a much higher sensitivity under the UV transillumination and is one of the most sensitive stains for detecting dsDNA in the agarose gel. In addition to the high sensitivity, the Novel Green Plus brings a more reliable and safer experience of use, since the stained gel can be visualized with the blue-light transilluminator, thus avoiding the risk of skin/eye damage as well as reducing the side effects of DNA modification caused by the UV light.

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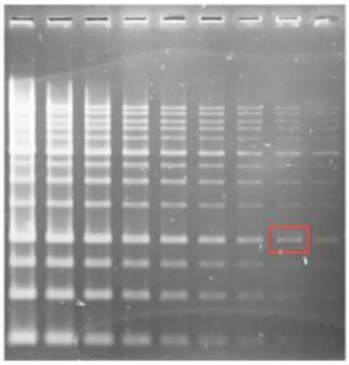

Fig.1a

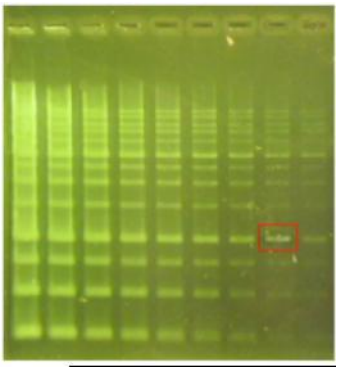

Fig.1b

Fig.1. 1KB DNA Ladder (250- 10k bp, GeneDireX) was 2X serial diluted (from 1 to 256 dilution, and the concentration of the red mark is 0.72 ng/ 5ul) and loaded in the 1% agarose gel. After electrophoresis, the gel was stained for 10 min with Novel Green *Plus* . The left- hand gel was observed with the UV 254 transilluminator and photographed by CCD camera (Fig.1a), and the right-hand gel was observed with the blue-light transilluminator (Fig.1b).

Spectral Characteristics

Novel Green Plus is excited at 497 nm but also shows a secondary excitation peak at 248 nm (Fig. 2a). After bound to DNA, the fluorescent emission of the Novel Green Plus is centered at 524 nm (Fig. 2b). These spectral characteristics enable this fluorescent dye to be compatible with a wide variety of gel reading facilities, including UV epi- and transilluminator, argon laser and mercury-arc lamp excitation gel scanners.

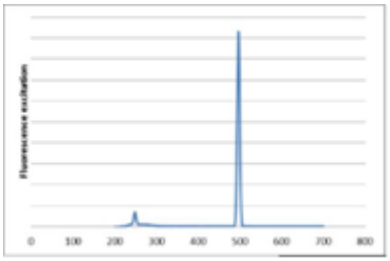

Fig. 2a. Fluorescence excitation spectra of the Novel Green *Plus*

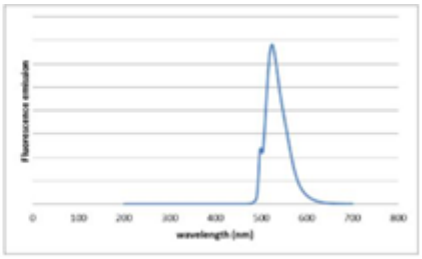

Fig. 2b. Fluorescence excitation and emission spectra of the Novel Green *Plus* bound to dsDNA.

Before opening, the vial must be warmed completely to room temperature to ensure that the dimethyl sulfoxide (DMSO) is completely thawed and that the solution is homogeneous. To avoid losing the stain, briefly centrifuge thawed stain in a microfuge to deposit the DMSO solution at the bottom of the vial. The stain may be divided into smaller aliquots and frozen for convenience. Smaller aliquots will thaw more quickly.

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Handling and Disposal

An independent laboratory has shown that Novel Green Plus stain is significantly less mutagenic than the ethidium bromide. However, we must caution that no data are available to address the mutagenicity or toxicity of the Novel Green Plus stain in humans. For the fact that this reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of the stain in compliance with local regulations.

Protocols

Post-Electrophoresis DNA Staining

1. Perform electrophoresis on an agarose gel.
   The Novel Green Plus is compatible with TAE (40mM Tris-acetate, 1mM EDTA, pH 8), TBE (89 mM Tris base, 89 mM boric acid, 1mM EDTA, pH 8), and TE (20mM Tris base, 1mM EDTA, pH 8) buffers.
2. Dilute the stock Novel Green Plus reagent with the 1:20,000 ratio.
   Stock stain can be diluted in the TE, TAE or TBE buffer.
   If the staining solution is diluted in water, it should be used within 24 hours.
   The buffered solution may increase the stability for this fluorescent staining dye.
3. Cover the gel with the staining solution and incubate at the room temperature for 10-30 minutes.
   Use a plastic container. Do not use a glass container since it will adsorb much of the dye in the staining solution.
   Protect the staining container from light by covering it with the aluminum foil or place it in the dark.
   Agitate the gel gently at the room temperature.
   Staining time will vary with the thickness of the gel and the agarose percentage.
   No destaining is required.
   The staining solution may be stored in the dark and at the low temperature for a week or more.
4. Photograph the gel with UV or blue-light transilluminator.
   It is important to clean the surface of the transillumuntor after / before each use with the deionized water and a soft cloth. Otherwise, fluorescent dyes may accumulate on the glass surface and cause a high fluorescent background.
   Video cameras and CCD cameras have a different spectral response than the black and white print film, thus it may not exhibit the same degree of sensitivity.

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Pre-Electrophoresis DNA Staining / In-Gel DNA Staining

1. Prepare molten agarose gel solution using your standard protocol.
2. Dilute Novel Green Plus 20,000X with the molten gel solution and mix well prior to being poured into the gel container.
   Cool the molten agarose gel until it can be handled by hand.
   The casted gel with Novel Green Plus Gel Stain will have a slight yellow appearance which is correlated to the dye strength.
   Casted gels are stable at 4℃ for 3 days in dark. After three days, the sensitivity will decrease daily.
3. Perform electrophoresis on an agarose gel (avoid light).
   The recommended voltage is 4–10 V/cm (distance between the anode and cathode). Avoid using high voltage during electrophoresis. High voltage causes excess heat and affects the dye adversely.
   During electrophoresis, the staining dye runs toward the anode, therefore DNA bands with smaller molecular weights may be weaker in intensity due to less staining dye.
4. Imagine the gel with the UV or blue-light transilluminator.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when staining DNA with the Novel Green Plus.

Problem	Cause	Solution
Low sensitivity	Wavelength may not be right.	Check the fluorescence excitation and emission wavelengths.
	Dilution ratio may not be right.	Check the dilution ratio in the 20,000- fold dilution.

Caution

Before opening, the vial should be warmed completely to the ambient temperature for ensuring that the DMSO is thawed thoroughly and that the solution is homogeneous.

The DMSO stock solution should be handled with applicable caution because DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of the stain in compliance with local regulations.

There is no data addressing the mutagenicity or toxicity of the fluorescent dye in humans. However, the fluorescent dye binds to nucleic acids; it should be recognized as a potential mutagen and used with appropriate care.