01494 Nancy-520 DNA Gel Stain

Application
Nancy-520 is a fluorescent stain for dsDNA on Agarose electrophoresis gels, with higher sensitivity than Ethidiumbromide and an easy, fast and robust staining procedure. In addition, Nancy-520 can be used to quantify dsDNA in solution.

Product Details

Spectral data:	λex = 520 nm / λem = 560 nm
Concentration:	Nancy-520 is provided as a 5000x stock solution in DMSO (5 mg/ml)
Content:	500 µl Nancy-520 is sufficient for 50 Agarose gels
Storage:	Protect from light; store at 4 °C
Sensitivity:	LOD: 0.5 ng/band dsDNA. Nancy-520 is less sensitive towards RNA.
Handling:	Warm to room temperature before opening. Do not expose to light unnecessarily.
Reuse:	Reuse of the dye is not recommended
Mutagenicity:	Ames test II has shown a lower mutagenic potential compared to SYBR Green I and a much lower mutagenic potential than Ethidium bromide.
Disposal:	Dispose of Nancy-520 in accordance with local regulations

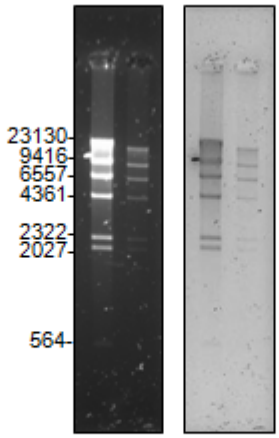

Figure 1. DNA Marker (Lambda DNA *Hind* III Digest) in 2 different concentrations, was separated on a 1 % Agarose gel, post-stained with Nancy-520 and imaged under 2 different conditions. Left: ex UV-Screen (300 nm) / em 590 nm bandpass filter/ CCD camera. Right: Laser-Scanner Fuji FLA-3000 / ex 532 nm / em 580 nm cut-off filter.

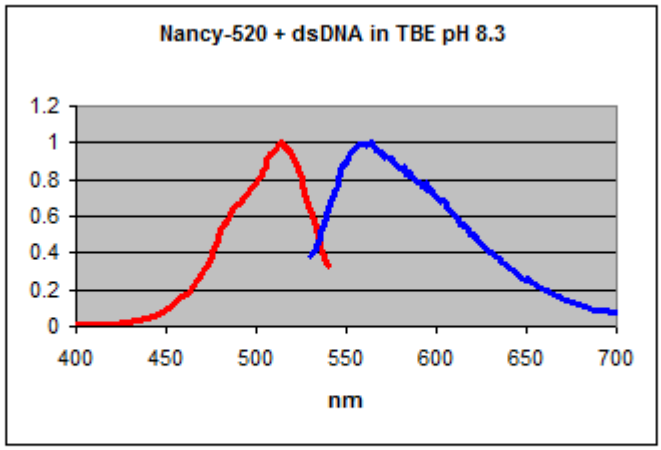

Figure 2. Normalized fluorescence excitation (red) and emission (blue) spectra of Nancy-520 in the presence of 1 µg/ml dsDNA, measured in a cuvette on a Varian Cary Eclipse Fluorescence Spectrophotometer in 2 ml TBE solution at pH 8.3. The excitation spectrum was measured at a fixed emission wavelength of 560 nm, and the emission spectrum was measured at a fixed excitation wavelength of 520 nm.

Standard Application: DNA staining

Post-staining protocol for DNA on Agarose Gels :

1. Run the gel
2. Prepare the staining solution by diluting the Nancy-520 stock solution 5000-fold (10 µl Nancy-520 in 50 ml electrophoresis buffer)
3. Immerse the gel after the run for 1h in 50 ml staining solution in the dark on a rocking table. Higher dye-concentrations will result in increased background staining.
4. Rinse the gel with 1x TBE (or TAE) buffer for 10 sec
5. Take a fluorescence image of the gel

Pre-staining protocol for DNA on Agarose Gels :

1. Prepare the gel from Agarose powder by adding the appropriate amount of Agarose to Electrophoresis buffer. Electrophoresis buffer can be TAE or TBE. Heat the mixture until it becomes homogenous.
2. Add 10 µl Nancy-520 stock solution to 50 ml of the agarose solution, while it is still liquid, but cooling down. Then pour the gel into the gel tray and insert the comb. Wait until the gel has become solid.
3. Mount the gel tray into the electrophoresis chamber
4. Cover the horizontal agarose gel with electrophoresis buffer
5. Mix the sample with gel loading solution
6. Load the sample onto the gel
7. Run the gel (e.g. 90 min at 90 V)
8. Take a fluorescence image of the gel directly after the run

Note: It is not possible to pre-stain a sample itself, before loading it onto the gel.

Detection
Detection is performed by illuminating the gel on a UV Screen or Dark Reader (Clare Chemical Research), and imaging the gel using a CCD-camera (e.g. Kodak Gel-Logic-100) with a 535 nm or 590 nm band-pass filter, or a Polaroid camera. Alternatively, a laser-scanner can be used (e.g. Fuji FLA-3000), with 473 nm excitation and 520 nm emission filters, or 532 nm excitation and 580 nm emission-filter. Other imaging systems are possible with the corresponding excitation sources and emission filter settings.
Try to minimize the exposure to light.

	UV Screen / Polaroid camera	UV Screen / 535 nm band pass filter / CCD camera	UV Screen / 590 nm band pass filter / CCD camera	Dark Reader / 590 nm band pass filter / CCD camera	Laser scanner ex 473 nm / 520 nm cut off filter	Laser scanner ex 532 nm / 580 nm cut off filter
Nancy-520	++	+++	+++	++	++	+++

Tested gel-systems
- Agarose gels (with TAE / TBE-buffer, pH 7.6 – 8.5)
- Polyacrylamide gels

Restriction Endonucleases
Many common restriction endonucleases are not inhibited by the presence of Nancy-520 bound to DNA, as tested exemplarily after band excision and gene elution.

Special application: DNA-Quantification in solution

Nancy-520 can be used to quantify dsDNA in solution.
This application can be performed in a 96-well plate with glass-bottom (read-out on Laser-Scanner or fluorescence microplate reader). For detection, we used a Fuji FLA-3000 Laser-Scanner with 532 nm excitation and 580 nm emission filter. Other imaging systems are possible with the corresponding excitation sources and emission filter settings.

1. DNA-standard: Use known concentrations of dsDNA as a standard
2. Dilute different concentrations of DNA-standard in 100 µl TE buffer pH 7.5
3. Dilute the unknown sample DNA in 100 µl TE buffer pH 7.5
4. Working solution: Dilute 4 µl Nancy-520 in 10 ml TE buffer pH 7.5
5. Add 100 µl working solution to each standard and each sample and mix
6. Measure the fluorescence immediately (total volume = 200 µl)
7. Compare the measured fluorescence values of the unknown sample with the DNA-standard values and calculate the concentration of the unknown sample on that basis

TE buffer: 10mM Tris / 1mM EDTA / pH 7.5. It is also possible to use TBE-buffer pH 8.3 instead. The samples must be free of ssDNA and other contaminants. There is a linear range between 0 and 2 µg/ml DNA.

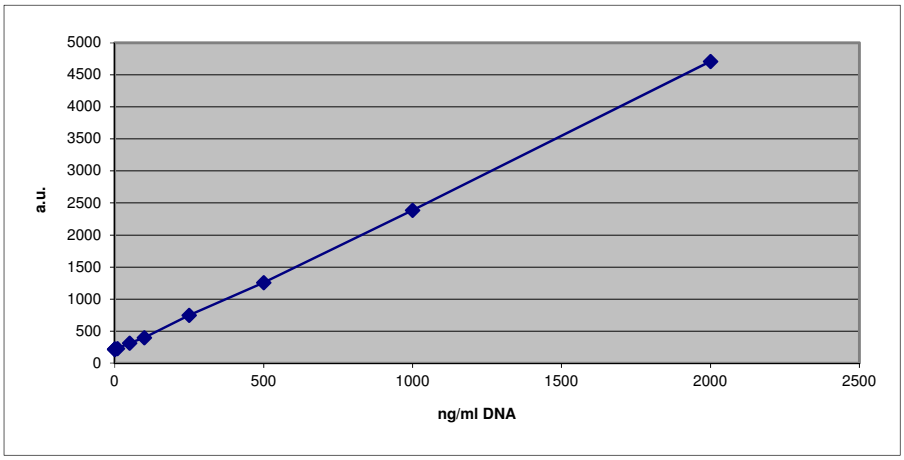

Figure 3. Quantification of DNA (Lambda DNA *Pst* I Digest) in solution using Nancy-520, performed in a 96-well plate. Detection was done on a Laser-Scanner (Fuji FLA-3000) with 532 nm excitation and 580 nm emission filter. Different concentrations of dsDNA in TE buffer pH 7.5 and their corresponding fluorescence values are shown. There is a linear range between 0 and 2 µg/ml DNA.

Related Products

Description	Cat. No.	Package size
Lambda DNA *Hind* III Digest	D9780	0.02 / 0.1 / 0.5 / 1 mg
Lambda DNA *Pst* I Digest	D1793	0.1 / 0.5 / 1 mg
TRIS borate – EDTA buffer substance	93309	1 / 5 l
TAE buffer	93295	0.1 / 1 / 5 l
Gel Loading Solution	G2526	5 ml
Agarose	A4679	50 / 100 / 500 g
GenElute™ Agarose Spin Columns	56500	70 ea

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.