infoPoint

Nucleic Acid Gel Stain with DNA-Dye NonTox

Ethidium Bromide (EtBr) is the most widely used DNA stain in molecular biology. However, due to safety and health concerns associated with exposure to this chemical, there has been increased interest in the use of alternative DNA stains that reduce health hazards and waste disposal processes. Those dyes have achieved interest among different labs, with the aim to reduce mutagenicity in DNA samples as well as being claimed as less hazardous and with low toxicity.

DNA-Dye NonTox is a non-toxic fluorescent reagent supplied in loading buffer, being a highly sensitive stain for the detection of double-stranded DNA (dsDNA). The dye produces instant visualization of DNA bands on gels upon blue light or UV illumination.

The perfect alternative to Ethidium Bromide

DNA-Dye NonTox is ideal in terms of environmental safety requiring a non-hazardous alternative to Ethidium Bromide. In addition, the dye included in DNA-Dye NonTox does not affect structure and integrity of DNA.

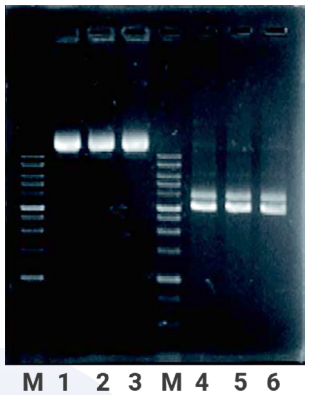
Supplied in 6X DNA Loading Buffer, DNA-Dye NonTox is used to prepare DNA markers and samples for loading on agarose or polyacrylamide gels. It contains three tracking dyes **Bromophenol Blue, Xylene Cyanol FF, and Orange G** for visually tracking the DNA migration during the electrophoresis process.

Spectral characteristics

Due to its spectral characteristics DNA-Dye NonTox is **compatible with most systems for gel visualization** and documentation. For highest sensitivity, choose **green detection filter** (approx. 537 nm) if possible. Excitation maxima of DNA-Dye NonTox are 300 nm (UV light) and 470 nm (blue light). Fluorescence emission of DNA-Dye NonTox bound to dsDNA is centered at 537 nm. The detection limit of DNA-Dye NonTox is **1-5 ng** DNA/band under optimal conditions, especially when blue light is used for excitation. Under UV light >10 ng DNA are typically well detectable.

Main Advantages

- As **sensitive** as Ethidium Bromide.
- **Non-Hazardous,** non-mutagenic and with low toxicity.
- **Low environmental impact.** No need of special measures with respect to waste management.
- DNA **structure and integrity not affected** so higher transformation rates are achieved.
- DNA-Dye NonTox does **not intercalate,** therefore, no variation in the migration behaviour is observed.



Agarose gel electrophoresis of DNA stained with DNA-Dye NonTox. DNA marker (M) and samples (1 - 6) were stained with **DNA-Dye NonTox,** separated by agarose gel electrophoresis and subsequently detected under UV light.

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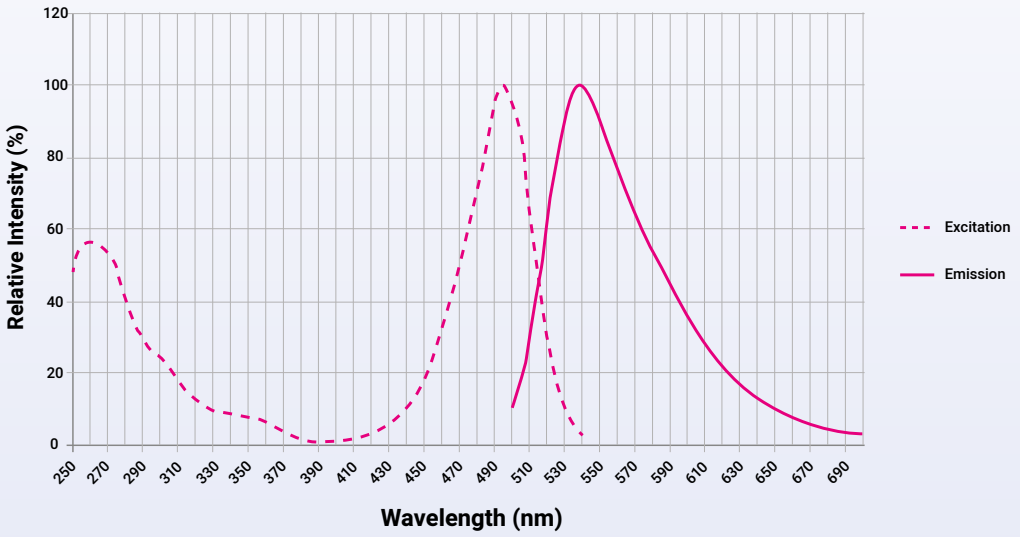
Fluorescence excitation/emission spectra of DNA-Dye NonTox nucleic acid gel stain bound to DNA



Short protocol

- Vortex DNA-Dye NonTox for 10 seconds prior to use.
- Dilute 1 part of DNA-Dye NonTox with 5 parts of DNA sample and mix*.
  Note: DNA-Dye NonTox must be added to DNA markers in order to visualize the ladder bands simultaneously with the sample after electrophoresis.
- Load sample and run according to standard procedures.
- After electrophoresis, remove gel and place on UV or a blue light transilluminator to immediately visualize bands.
  *DNA-Dye NonTox is a ready-to-use solution supplied as a 6X Loading Dye. No de-staining is required, and it produces low background noise.

Comparison with other DNA Gel Dyes

	DNA-Dye NonTox	Ethidium Bromide	SYBR Safe	GelRed	Methylene Blue	Crystal Violet
Protocol	Added to DNA sample and marker.	Can be used in the gel at or as a post-stain at a concentration of 0.5 mg/L.	Used as an in-gel stain only. It is supplied in ready-made buffers.	Can be used as post stain or in-gel stain. It is supplied in ready-made buffers	Post stain only, in 0.025% (w/v) methylene blue in water.	Used in gels at a concentration of around 1.2 mg/mL
Detection	**Compatible with most systems for gel visualization.**	UV transilluminator	Blue light transilluminator.	UV transilluminator.	Visible light.	Visible light.
Sensitivity	As sensitive as ethidium bromide: bands of 1-5 ng should be detectable.	Can detect bands of 1-5 ng.	As sensitive as ethidium bromide: bands of 1-5 ng should be detectable.	Bands of 0.25 ng	Bands of 500 ng	Bands of 50-200 ng
Toxicity	Non-toxic, nonmutagenic	Toxic, mutagen, teratogen and carcinogen according to a variety of tests.	Less mutagenic than ethidium bromide but its acute toxicity is higher.	Less mutagenic than ethidium bromide.	Non-mutagenic. Toxic if ingested.	Less mutagenic than ethidium bromide.
Migration behaviour	It attaches to DNA strands, but **does not intercalate.** Variations in the migration behaviour between samples and markers are rarely observed.	Ethidium bromide intercalates between the DNA strands.	As a gel stain, the dye migrates in the opposite direction of DNA, and bottom of gel may have lower dye concentration.	The migration in agarose gel electrophoresis of DNA fragments is shifted to a higher molecular size when using GelRed to stain the DNA.	No effect, as it is a post stain dye.	Combination with bromophenol blue can alter the migration of DNA in the presence of crystal violet.

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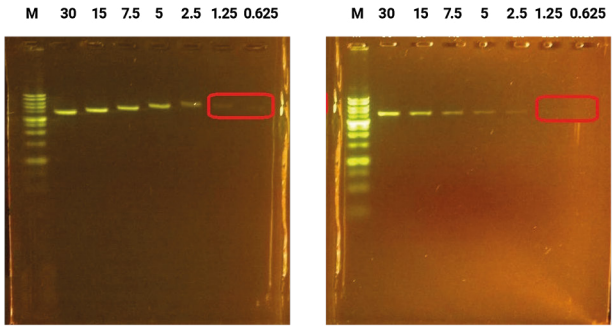
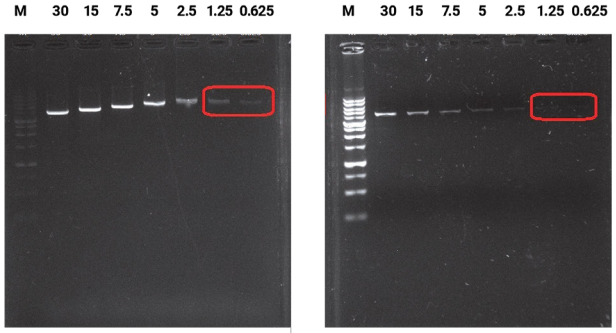
Sensitivity > 1 ng. More sensitive than ethidium bromide (1 ng) and SYBR Safe (3 ng)



Concentration: ng/µl
Volume: 6 µl / lane
LED wavelength: 470 nm
M: 1 kb DNA ladder

Sensitivity Comparison under LED Transilluminator: DNA-Dye NonTox (left) and SYBR Safe (right).



Concentration: ng/µl
Volume: 6 µl / lane
UV wavelength: 300 nm
Exposure: 1 min.
M: 1 kb DNA ladder

Sensitivity Comparison under UV Transilluminator: DNA-Dye NonTox (left) and SYBR Safe (right).

Assessment of Mutagenic Potential

	Controls		Dilution Factor of substance DNA-Dye NonTox				
	Negative control group (D-PBS)	Positive control group (4NOP)§	1X	2X	4X	8X	16X
Mean bacterial population ± SD	19 ± 3	1325 ± 247	35 ± 2	19 ± 7	22 ± 2	21 ± 1	19 ± 4
Mutagenicity*	-	69.73	1.84**	1.02	1.14	1.12	0.98

Table 1: Ames test/Mutagenicity test results using bacterial strain TA-98 (S9-deficient experiment group) for testing DNA Dye NonTox in comparison to Phosphate buffered saline (PBS) as negative control and 4NOP (4-nitro-o-phenylendiamine) as positive control group (n=3).

	Controls		Dilution Factor of substance DNA-Dye NonTox				
	Negative control group (D-PBS)	Positive control group (SA)§	1X	2X	4X	8X	16X
Mean bacterial population ± SD	14 ± 3	508 ± 17	11 ± 6	12 ± 2	13 ± 3	11 ± 6	18 ± 1
Mutagenicity*	-	36.31	0.79	0.88	0.93	0.81	1.29

Table 2: Ames test/Mutagenicity test results using bacterial strain TA98 (S9-deficient experiment group) for testing DNA-Dye NonTox in comparison to Phosphate buffered saline (PBS) as negative control and SA (Sodium azide) as positive control group (n=3).

* Mutagenicity = Testing substance / negative control group (§ Indication of significance ($p < 0.05$))
**The mean bacterial population of the testing substance DNA-Dye NonTox was 1.84-fold greater than that for the negative control group, which was <2- fold, but p value was 0.001 and exhibited significance.

Nucleic Acid Gel Stain with DNA-Dye NonTox

IP-038EN;202302

Product code	Product name	Pack size
A9555,1000	DNA-Dye NonTox	1 mL

Storage: 2 – 8°C, protected from light
Shelf life: approx. 12 months

Related products

Product code	Product name	Pack sizes
A8963,0100	Agarose Basic	100 g
A8963,0250		250 g
A8963,0500		500 g
A8963,1000		1 kg
A2114,0100	Agarose low EEO (Agarose Standard)	100 g
A2114,0250		250 g
A2114,0500		500 g
A7089,0100	DNA/RNA-ExitusPlus™	100 mL
A7089,0500		500 mL
A7089,1000RF		1 L
A7089,2500RF		2.5 L
A7409,0100	DNA/RNA-ExitusPlus™ IF	100 mL
A7409,0500		500 mL
A7409,1000RF		1 L
A7409,2500RF		2.5 L
A7409,5000		5 L
A3778,0010	DNase I	10 mg
A3778,0050		50 mg
A3778,0100		100 mg
A3778,0500		500 mg
A4972,0010	Lysozyme for molecular biology	10 g
A0889,0100	Phenol equilibrated, stabilized : Chloroform : Isoamyl Alcohol 25 : 24 : 1	100 ml
A0889,0500		500 ml
A3830,0025	Proteinase K	25 mg
A3830,0100		100 mg
A3830,0220		220 mg
A3830,0500		500 mg

Product code	Product name	Pack sizes
A7153,0500	RNase-ExitusPlus™	500 mL
A7153,1000RF		1 L
A7153,2500RF		2. 5 L
A3832,0050	RNase A (DNase-free)	50 mg
A3832,0250		250 mg
A3832,0500		500 mg
A1691,1000	TAE buffer (50X)	1 L
A4051,0100	TRItidy G™	100 mL
A4051,0200		200 mL

References
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Haines, A. M. et al., (2015). Properties of nucleic acid staining dyes used in gel electrophoresis. Electrophoresis, 36(6), 941-944.
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