



## 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

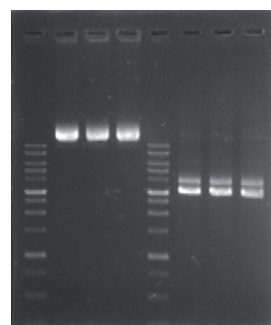
Due to its spectral characteristics DNA-Dye NonTox is **compatible with most systems for gel visualization** and documentation. For highest sensitivity, choose **green detection filter** (approx. 537 nm) if possible. Excitation maxima of DNA-Dye NonTox are 300 nm (UV light) and 470 nm (blue light). Fluorescence 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

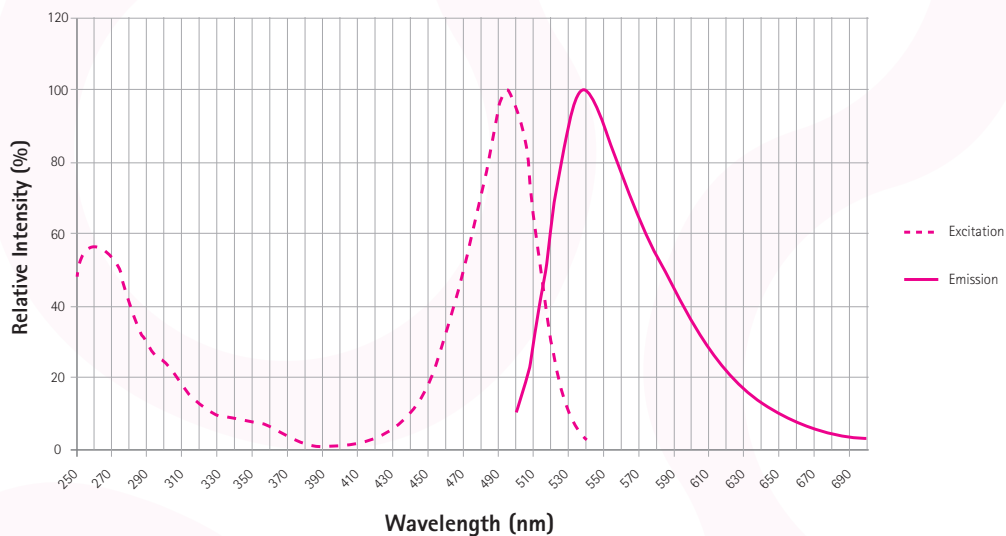
- As **sensitive** as Ethidium Bromide.
- **Non-Hazardous**, non-mutagenic and with low toxicity.
- **Low environmental impact**. No need of special measures with respect to waste management.
- DNA **structure and integrity not affected** so higher transformation rates are achieved.
- DNA-Dye NonTox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



M 1 2 3 M 4 5 6

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.

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



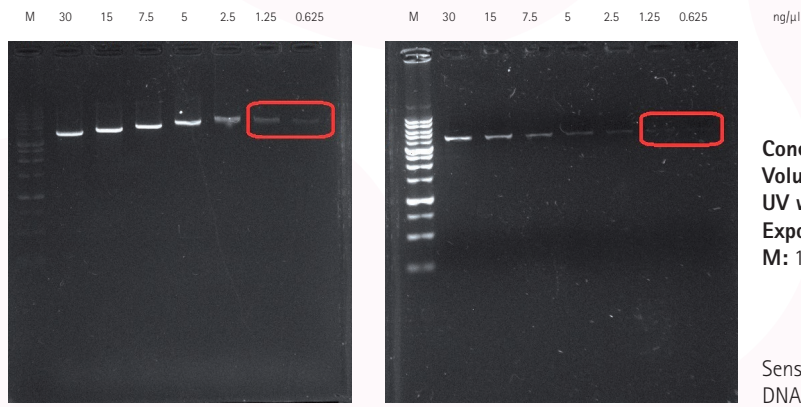
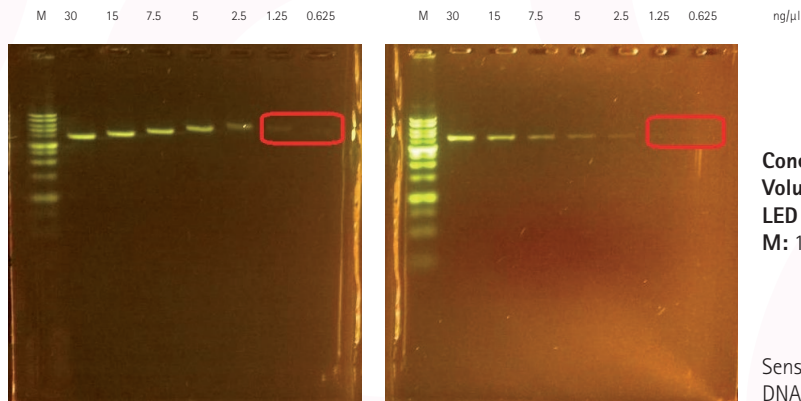
### Short protocol

- 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	<b>Compatible with most systems for gel visualization.</b>	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, non-mutagenic.	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 <b>does not intercalate</b> . 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.

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



## Assessment of Mutagenic Potential

	Controls		Dilution Factor of substance DNA-Dye NonTox				
	Negative control group (D-PBS)	Positive control group (4NOP)S	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-phenylenediamine) as positive control group (n=3).

	Controls		Dilution Factor of substance DNA-Dye NonTox				
	Negative control group (D-PBS)	Positive control group (SA)S	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 (S 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.



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Description	Code	Package
DNA-Dye NonTox	A9555,1000	1 ml

Storage: 2 – 8°C, protected from light

Shelf life: approx. 12 months

## Related Products

Description	Code	Package
Agarose Basic	A8963,0100	100 g
	A8963,0250	250 g
	A8963,0500	500 g
	A8963,1000	1 kg
Agarose low EEO (Agarose Standard)	A2114,0100	100 g
	A2114,0250	250 g
	A2114,0500	500 g
DNA-ExitusPlus™	A7089,0100	100 ml
	A7089,0500	500 ml
	A7089,1000RF	1 L
	A7089,2500RF	2.5 L
DNA-ExitusPlus™ IF	A7409,0100	100 ml
	A7409,0500	500 ml
	A7409,1000	1 L
	A7409,1000RF	1 L
	A7409,2500RF	2,5 L
	A7409,5000	5 L
DNase I	A3778,0010	10 mg
	A3778,0050	50 mg
	A3778,0100	100 mg
	A3778,0500	500 mg
Lysozyme for molecular biology	A4972,0001	1 g
	A4972,0010	10 g
Phenol equilibrated, stabilized : Chloroform : Isoamyl Alcohol 25 : 24 : 1	A0889,0100	100 ml
	A0889,0250	250 ml
	A0889,0500	500 ml
Proteinase K	A3830,0025	25 mg
	A3830,0100	100 mg
	A3830,0500	500 mg
RNase-ExitusPlus™	A7153,0500	500 ml
	A7153,1000	1 L
	A7153,1000RF	1 L
	A7153,2500RF	2,5 L
RNase A (DNase-free)	A3832,0050	50 mg
	A3832,0250	250 mg
	A3832,0500	500 mg
TAE buffer (50X)	A1691,0500	500 ml
	A1691,1000	1 L
TRItidy G™	A4051,0100	100 ml
	A4051,0200	200 ml



### References

- Hunter, S. B. et al., (2005). Journal of Clinical Microbiology, 43(3), 1045-1050.  
 Huang, Q. (2010). Clin. Lab, 56, 149-152.  
 Haines, A. M. et al., (2015). Properties of nucleic acid staining dyes used in gel electrophoresis. Electrophoresis, 36(6), 941-944.  
 Lalchandama, K. (2016). Sciencevision.

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