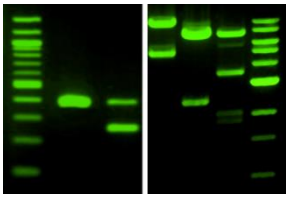
Technical Info

Well advised with Roth.



# Gel Staining of Nucleic Acids

## Gel Staining Solutions

Nucleic acid dyes for agarose and acrylamide gels, for laboratories and practical applications. We offer you high-quality stains with high sensitivity (silver stains), standard stains (ethidium bromide) in user-friendly dropper bottles, and non-toxic green-fluorescent dyes for blue or UV light. The following table provides an overview of the various stains and their respective sensitivity levels.

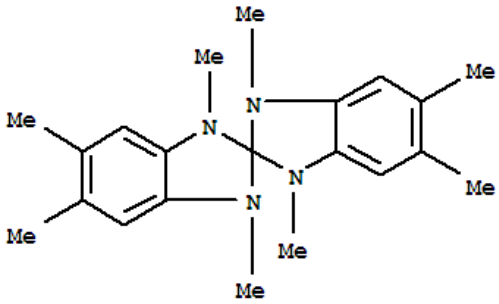
## Summary: Staining of Nucleic Acid in Gels

| Staining | Art. No. | Sensitivity | Description |
|---|---|---|---|
| ROTI®Black N | N769 | <0,1 ng/mm² | **Silver staining** of DNA polyacrylic amide gels, visible under white light. |
| ROTI®Black NSeq | P081 | <0,1 ng/mm² | **Silver staining** of DNA sequencing gels, visible under white light. |
| ROTI®Load DNAstain | 5783, 5784, 6472 | 1,5 ng/mm² | Sensitive, **non-toxic fluorescent staining** of DNA (excitation 320 nm and 490 nm/blue light). Combined with gel loading buffer for fragments >500 bp (DNAstain 1), 100-2000 bp (DNAstain 2), or <500 bp (DNAstain 3). |
| ROTI®GelStain | 3865 | 1,5 ng/mm² | Sensitive **fluorescent staining** of DNA (excitation: 290-320 nm wave length). Non-toxic, noncarcinogenic. |
| ROTI®GelStain Red | 0984 | 0.3 ng/mm² | Sensitive **fluorescent staining** of DNA (excitation: 300 nm wave length and 530-550 nm wave length). Non-toxic, noncarcinogenic |
| Ethidium bromidesolution | 2218 (1 %)<br>HP46 (0,5 %)<br>HP47 (0,025 %) | 1,5 ng/mm² | Quick standard staining of nucleic acid, **fluorescent** (excitation: 254-360 nm wave length). |
| ROTI®Methylene blue staining concentrate | 0648 | 10 ng/mm² | Reversible, **non-toxic blue staining** of DNA, visible under white light. |

# ROTI®GelStain

## Mechanism

- Ready-to-use staining mixture prepared from a variety of reagents, dye component: orange-red to brown powder.
- Benzimidazoles typically bind to the minor groove of helical nucleic acid. No intercalation takes place.
- Fluorescence staining, requires UV-excitation.
- Excitation maximum (DNA bound): 290-320 nm
  Emission maximum (DNA bound): 515 nm




## Hazard

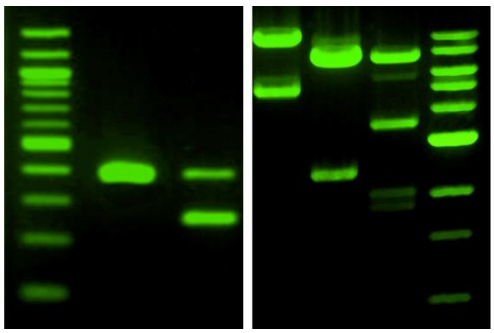
None known. Not membrane permeable.

## Applications

Suitable for all agarose gels.

Not suitable for staining of PAA gels.

Sensitivity: 1,5 ng/mm2 (0.2 ng/band).



## Staining

- Staining is reversible
- Addition to gel (liquid agarose) [approx. 5 µl/100 ml]
- Addition to running buffer [approx. 25 µl/100 ml]
- Staining after gel run [approx. 25 µl/100 ml]
- Addition to loading buffer /nucleic acid not recommended

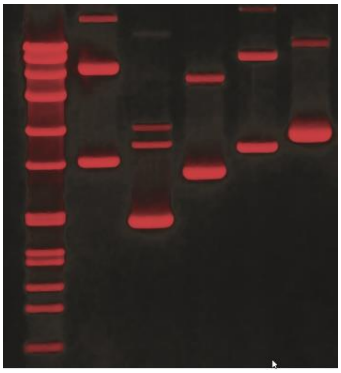
## Please note

- When applied during the gel run: Nucleic acid that has been loaded with ROTI®GelStain shows slightly altered running behaviour when compared to free nucleic acid.
- Staining depends on secondary and tertiary structure of the nucleic acids. Due to the optimal formation of the minor groove, longer fragments of linear dsDNA are stained more efficiently than short fragments (below 500 bp), ssDNA, short RNA or cyclic dsDNA.
- DNA quantitation in the gel is possible, as long as the both DNAs compared are having the same secondary and tertiary structure. For instance, cyclic DNA should only be quantified by comparison with a cyclic DNA standard.

# ROTI®GelStain Red

## Mechanism

- ROTI®GelStain Red is a red, DNA intercalating dye.
- Fluorescence staining, requires UV- or blue light excitation.
- Excitation maximum (DNA bound): 300 nm (UV light) and 530-550 nm (blue light)
  Emission maximum (DNA bound): 630 nm



## Hazard

None known. Not membrane permeable.

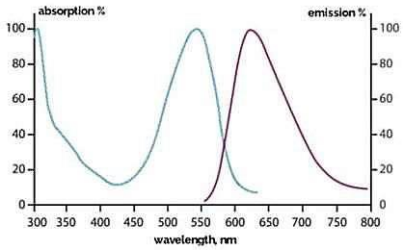
## Applications

Suitable for all agarose and PAA gels.

Suitable for staining of dsDNA, ssDNA and RNA.

Usable with the same filters as ethidium bromide.

Sensitivity: 0.1 - 0.3 ng/band nucleic acid.




## Staining

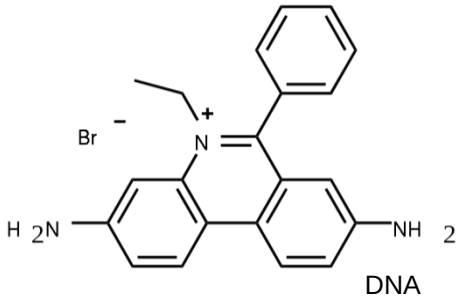
To be used at 1x conc. in agarose or PAA gel + 0.5x conc. in running buffer

- Addition to gel (liquid agarose) [approx. 5 µl/100 ml]
- Addition to gel (liquid PAA) [approx. 5 µl/100 ml]
- Addition to running buffer [approx. 2.5 - 3 µl/100 ml]
- Addition to loading buffer/nucleic acid is not recommended.
- Post-run staining is not recommended.
- Staining in principle reversible, but hard to remove

## Please note

- When applied during the gel run: Nucleic acid that has been loaded with ROTI®GelStain Red shows slightly altered running behaviour when compared to free nucleic acid.
- For cloning applications: Keep UV exposure as low as possible, preferably work only with blue light.
- DNA quantitation in the gel is possible. As long as the DNA ladder used as a reference for quantification and the DNA samples to be quantified are run and stained on the same gel with the same stain, the ladder bands of known quantity can be used as references for approximate in gel DNA band quantification.

# Ethidium bromide

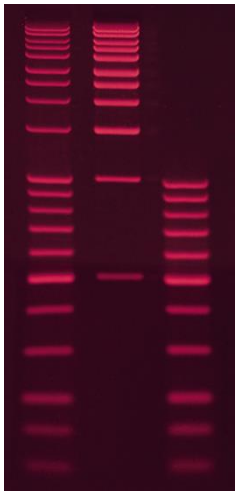



## Mechanism

- Intercalation between base pairs of nucleic acids
- Very strong binding to dsDNA
- Fluorescence staining, requires UV-excitation
- Excitation maxima
  bound: 330 nm, 500 nm (Excitation possible at 260 - 350 nm)
  unbound: 210 nm, 285 nm, 470 nm
  Emission maximum: 605 nm

## Hazard

Dangerous up to toxic, mutagenicity may not completely be excluded.



## Applications

Suitable for all agarose gels and PAA gels.

Sensitivity: 1.5 ng/mm$^2$ (0,2 ng/band).

## Staining

- Addition to loading buffer /nucleic acid [approx. 2 µg/ml]
- Addition to gel (liquid agarose) [approx. 0,2 µg/ml]
- Staining after gel run [approx. 2 µg/ml, 10 mins,
  optional: destaining for 15 mins.]
- Staining in principle reversible, but hard to remove

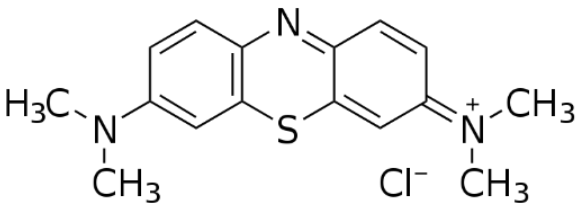
## Please note

- When applied during the gel run: Nucleic acid that has been loaded with ethidium bromide shows slightly altered running behaviour when compared to free nucleic acid
- Linear dsDNA will be stained more intensely than ssDNA, RNA, or cyclic dsDNA
- Quantitation of DNA in the gel is possible if compared DNA has identical secondary and tertiary structure; e.g.: compare cyclic DNA only with equally cyclic DNA

# Methylene blue

## Mechanism

- Ionic binding to phosphoric acid of nucleic acids results in weak binding
- Blue staining, visible in white light




## Hazard

None known.

## Applications

Only applicable as staining solution after the gel run.

Only suitable for thin agarose gels of up to approx. 1,5 % agarose and PAA gels.

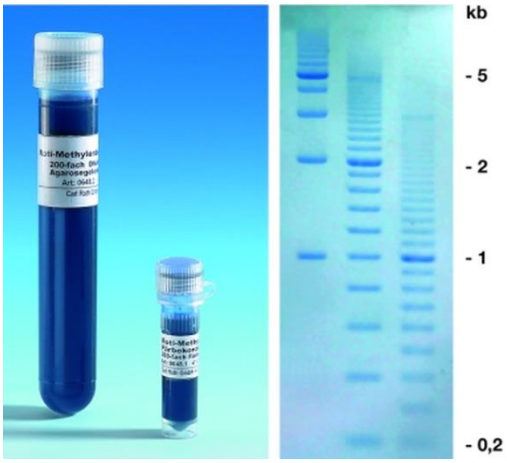
Sensitivity: 10 ng/mm$^2$ (1,5 ng/band).

## Staining

- Staining for 10 - 30 min.
- Destaining necessary for 15 min. to over night
- Reversible staining, easy to remove

## Please note

- Methylene Blue Staining Concentrate contains a solvent, causing
- DNA to precipitate after addition to the loading buffer
- dsDNA/RNA will be stained more intensely than ssDNA/RNA

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