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Gel Staining of Nucleic Acids

Gel Staining Solutions

Nucleic acid dyes for agarose and polyacrylamide (PAA) gels, for laboratories and practical applications. We offer you high-quality stains with high sensitivity (silver stains or SYBR®Green), standard stains (ethidium bromide) in user-friendly dropper bottles, and non-toxic 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
Ethidium bromide solution	2218 (1 %) HP46 (0,5 %) HP47 (0,025 %)	0.5 - 5 ng/gel band	• Quick standard **fluorescent staining** of nucleic acids . • Excitation: 254-360 nm wave length. • Suitable for In-Gel and Post-Run staining
ROTI®Black N	N769	<0.01 ng/gel band	• **Silver staining** of DNA polyacrylamide gels • Visible under white light. • Suitable for Post-Run Staining
ROTI®Black NSeq	P081	<0.01 ng/gel band	• **Silver staining** of DNA sequencing gels • Visible under white light. • Suitable for Post-Run Staining
ROTI®GelStain	3865	<0.3 ng/gel band	• Sensitive **fluorescent staining** of DNA & RNA in agarose or PAA gels. • Excitation: 302 nm and 490 nm wave length. • Non-toxic, non-carcinogenic. • Suitable for in-Gel staining
ROTI®GelStain Red Eco	223C	>0.1 ng/gel band	• Sensitive **fluorescent staining** of DNA & RNA in agarose or PAA gels. • Excitation: 300 nm and 500 nm wave length. • Non-toxic, noncarcinogenic • Suitable for in-Gel and Post-Run staining
ROTI®Methylene blue staining concentrate	0648	10 ng/gel band	• Reversible, non-toxic **blue staining** of DNA • Visible under white light. • Suitable for Post-Run staining
SYBR®Green	1CN2 1CN5* 1CN6* 1CN7* *Mix with Loading Dye	0.01 ng/gel band	• Very sensitive **fluorescent** staining of DNA in agarose or PAA gels. • Excitation: 254 nm and 495 nm wave length. • Suitable for In-Sample staining

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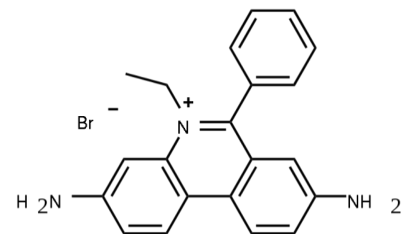
Ethidium bromide

Applications

- Suitable for In-Gel staining, Post-Run staining and In-Sample staining of all agarose and PAA gels.
- Sensitivity: 0.5 – 5 ng/gel band

Mechanism



- Intercalation* between base pairs of nucleic acids
- Very strong binding to dsDNA
- Fluorescence staining, requires UV-excitation
- Excitation maxima (DNA bound) 330 nm and 500 nm

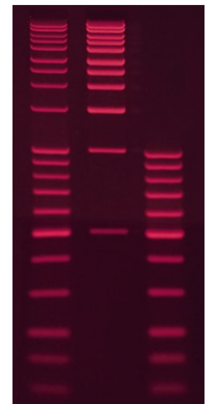
  (Excitation possible at 260-350 nm)
  Emission maximum: 605 nm

Hazard

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

Safety

Positive in the Ames-Test*→ potentially mutagenic



Staining

- Addition to loading buffer /nucleic acid [approx. 2 µg/ml]
- Addition to gel (liquid agarose) [approx. 0,2 µg/ml]
- Post-run staining [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

*For information regarding the relationship between DNA intercalation and mutagenicity or the investigation of mutagenicity please look at p.8

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ROTI®Black N and ROTI®Black NSeq

Applications

- Suitable for Post-Gel staining of PAA gels (ROTI®Black N) and Sequencing gels (ROTI®Black NSeq)
- Suitable for staining of dsDNA, ssDNA and RNA.
- Sensitivity: <0.01 ng/gel band

Mechanism

- Positively charged silver ions attach to negatively charged DNA
- Addition of formaldehyde reduces silver solution to metallic silver
  ➔ visible as black under white light

Hazard

Contains dangerous and toxic reagents (formaldehyde), handle with care

Safety

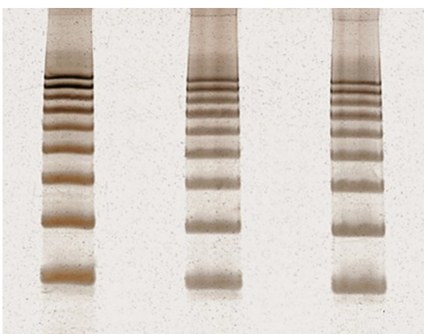
Safer alternative to radioactive visualization

Staining

- Ultra-sensitive staining of nucleic acids
- Only post-run staining of PAA-gels
- Staining is not reversible

Please note

- Please maintain the exact times for reproducible results
- More sensitive than organic stains
- Staining is stable for several weeks
- DNA quantitation in the gel is possible



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ROTI®GelStain

Applications

- Suitable for In-Gel staining of all agarose gels and PAA gels
- Suitable for staining of dsDNA, ssDNA and RNA.
- Sensitivity: 0.1 - 0.3 ng/gel band

Mechanism

- ROTI®GelStain is a nucleic acid intercalating dye*
- Fluorescence staining, requires UV excitation or blue light for visualisation
- Excitation maximum (DNA bound): 302nm and 490nm
  Emission maximum (DNA bound): 520nm

Hazard

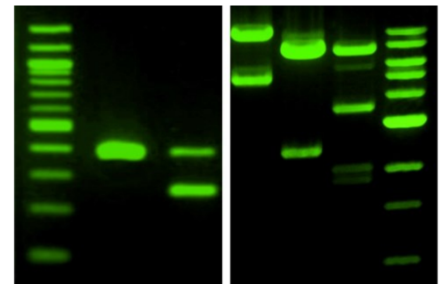
None known.

Safety

- Less mutations than ethidium bromide in the Ames-Test*
- Tests negative in the mouse marrow chromophilus erythrocyte micronucleus test and the mouse spermato-cyte chromosomal aberration test*

Staining

- Staining is reversible, but hard to remove
- Emits green fluorescence when bound to dsDNA,ssDNA or RNA
- Addition to liquid gel [5µl/100ml] and to running buffer [5µl/100ml]
- Addition to loading buffer/sample is not recommended
- Post-run staining is not recommended



Please note

- For downstream cloning applications we recommend ROTI®GelStain Red (0984), as ROTI®GelStain might disturb ligation reactions
- 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 base stack intercalating mechanism 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 both DNA samples compared have the same secondary and tertiary structure. For instance, cyclic DNA should only be quantified by comparison with a cyclic DNA standard.

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ROTI®GelStain Red Eco

Applications
- Suitable for In-Gel and Post-Run staining of all agarose and PAA gels.
- Suitable for staining of dsDNA, ssDNA, RNA and short DNA
- Sensitivity: >0.1 ng/gel band

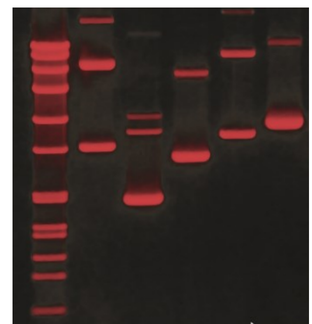
Mechanism
- ROTI®GelStain Red Eco is a nucleic acid DNA intercalating dye*
- Fluorescence staining requires UV- or blue light excitation.
- Also visible under blue LED
- Excitation maximum (DNA bound): 300 nm (UV light) and 500 nm (blue light)
  Emission maximum (DNA bound): 600 nm

Hazard

None known.

Safety
- Less mutations than ethidium bromide in the Ames-Test*

Staining
- Staining is in principle reversible, but hard to remove
- Emits orange fluorescence when bound to dsDNA, ssDNA or RNA
- For In-Gel staining: Addition to liquid gel [10µl / 100ml]
- For Post-Run Staining: Addition to electrophoresis buffer [30µl / 100µl]
- Addition to loading buffer/sample is not recommended



Please note
- When applied during the gel run: Nucleic acid that has been loaded with ROTI®GelStain Red Eco 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.

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Methylene blue

Applications

- Suitable for Post-Staining of thin agarose (1.5 % agarose) and PAA gels.
- Suitable for reversible staining of Southern- and Northern Blots
- Suitable for staining of dsDNA, ssDNA and RNA.
- Sensitivity: 10 ng/gel band

Mechanism

- Positively charged ammonium group of methylene blue binds to negatively charged DNA
- Ionic binding to phosphoric acid of nucleic acids results in weak binding
- Blue staining, visible in white light

Hazard

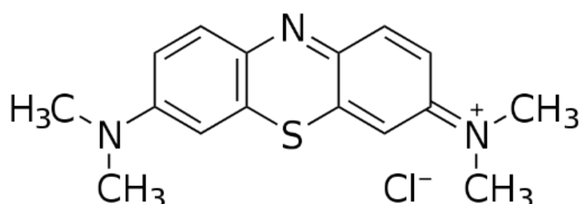
None known.

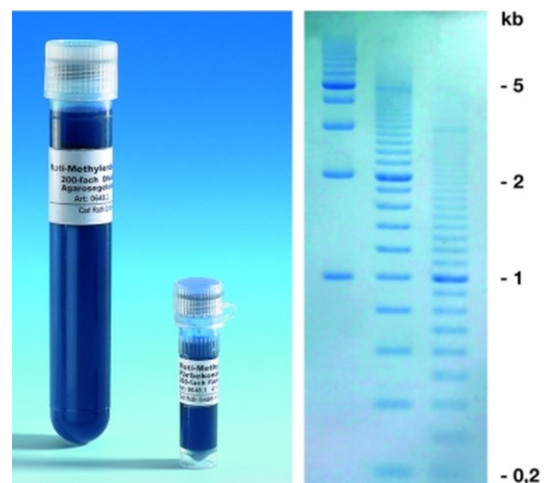
Staining

- 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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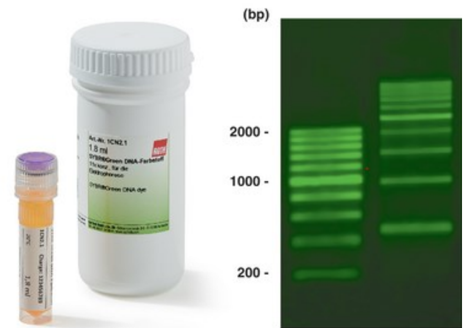
Technical Info

SYBR®Green



Applications

- Suitable for In-Sample staining of all agarose and PAA gels
- Suitable for staining of dsDNA
- Sensitivity: 0.1 ng/gel band

Mechanism

- Intercalates in the minor groove of dsDNA or between the base stacks (smaller amount)[1]
- Fluorescence staining requires UV- or blue light excitation.
- Excitation maximum (DNA bound): 254 nm (UV light) and 495 nm (blue light)
  Emission maximum (DNA bound): 521 nm

Hazard

The SYBR®Green staining solution is very low concentrated (<0.1 %) and therefore not classified as mutagenic. However, we recommend to handle it with caution, as carcinogenicity and mutagenicity cannot be completely excluded.

Safety

- Less mutations than ethidium bromide in the Ames-Test[2*]
- More cytotoxic than ethidium bromide[2]

Staining

- Particularly mild, does not affect DNA integrity
- Emits green fluorescence when bound to DNA
- High DNA-affinity → small amount of dye is needed for a strong signal
- Reversible staining, easy to remove
- Post-Run staining and In-Gel staining are not recommended due to the large amount of dye that would be required.

Please note

- Nucleic acid that has been loaded with SYBR®Green shows slightly altered running behaviour when compared to free nucleic acid. **Tip:** Use prestained DNA-Ladder (1CN8.1 or 1CN9.1) as reference.
- 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.

* For information regarding the relationship between DNA intercalation and mutagenicity or the investigation of mutagenicity please look at p.8

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DNA Intercalation and Mutagenicity

DNA Intercalation

A common question in the safety of DNA stains is, whether DNA stains do intercalate in the DNA or if they just bind to certain DNA structures (such as the minor groove). The reason behind this question is, that it is believed that DNA intercalating dyes have a stronger potential for being mutagenic and therefore carcinogenic. Indeed, dyes that intercalate into DNA can disturb the replication machinery, thus resulting in mutations[3,4]. However, this is not the only criterion regarding the mutagenicity of a dye. Even dyes such as Hoechst, which only bind in the minor groove of DNA, must first be removed before DNA replication and transcription, as mutations can otherwise occur[5].

To limit the genotoxic danger that arises from the interaction of DNA and fluorescent dyes, there are two additional conditions that restrain this danger: **Membrane permeability** as a physical barrier and the **sensitivity of the dye**, as lower concentrations are needed, thus reducing the quantity of DNA damage.

Methods for the investigation of mutagenicity

The mutagenicity rate of certain DNA stains can be tested by some common methods, including the **Ames test** or the ***in vivo* micronucleus assay** in mouse bone marrow[6,7].

In the **Ames test** auxotrophic *Salmonella typhimurium* bacteria strains which carry mutations in genes involved in histidine synthesis, are cultivated on histidine-free medium containing different concentrations of the potential mutagen. The strains can only grow on the media, if they get a reverse mutation in the gene for histidine synthesis, as this restores their prototrophy. If an enhanced number of cells is growing in this assay, it is a clear indication, that the substance has the potential to be mutagenic[6].

However, since the substances are tested for their mutagenic potential in mammals, which in turn could lead to cancer, the Ames test alone is not sufficient, as eukaryotes have a completely different metabolism than prokaryotes. If eukaryotic cells are exposed to a substance that is classified as non-mutagenic according to the Ames test, it may be that new products are generated by metabolism, which then very well have a mutagenic effect on the genetic material. For this reason the potential mutagens have also to be tested in eukaryotic systems, for example with the ***in vivo* micronucleus test**. In this cytogenetic test animals or cell cultures are treated with genotoxic compounds. Subsequently the polychromatic erythrozytes are examined, as during their development from bone marrow to polychromatic erythrozytes the nucleus is extruded. Upon DNA damage micronuclei can be formed, and these then remain in the otherwise anucleated cytoplasm and can be detected with microscopy. If an increase of micronucleated polychromatic erythrozytes is observed, this is an indication for induced chromosome damage[7].

If these mutagenicity tests are negative, the potential for mutagenicity is significantly reduced. However, to completely rule out potential mutations and prevent the development of cancer, it is advisable to **wear gloves** when handling DNA dyes, especially if they are used frequently.

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[1]**Zipper H, Brunner H, Bernhagen J, Vitzthum F**. Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. *Nucleic Acids Res.* **2004** Jul 12;32(12):e103

[2]**Singer VL, Lawlor TE, Yue S.** Comparison of SYBR® Green I nucleic acid gel stain mutagenicity and ethidium bromide mutagenicity in the Salmonella/mammalian microsome reverse mutation assay (Ames test) Mutat. *Res. Genet. Toxicol. Environ. Mutagen.,* **439** (1), pp. 37-47 (1999)

[3]**Biebricher A, Heller I, Roijmans R.** ***et al.*** The impact of DNA intercalators on DNA and DNA-processing enzymes elucidated through force-dependent binding kinetics. *Nat Commun* **6**, 7304 (2015)

[4]**Hurley, L.** DNA and its associated processes as targets for cancer therapy. *Nat Rev Cancer* **2**, 188–200 (2002).

[5]**Durand RE, Olive PL.** Cytotoxicity, Mutagenicity and DNA damage by Hoechst 33342. *J Histochem Cytochem.* **30**(2):111-6 (1982)

[6]**Ames BN, Mccann J, Yamasaki E.** Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutat Res.* (**6**) 347-64 (1975)

[7]**Hayashi, M**. The micronucleus test—most widely used *in vivo* genotoxicity test—. *Genes and Environ* **38**, 18 (2016).

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