Technical Bulletin

Characteristics and Usage of RedSafe Nucleic Acid Staining Solution (20,000X)

Abstract

RedSafe Nucleic Acid Staining Solution (20,00x) is a new and safe alternative favourably reviewed by many researchers against ethidium bromide(EtBr) which is a generally accepted traditional method when dyeing nucleic acid in Agarose gel. This manual aims to secure the best result by guiding right usage and the characteristics of RedSafe as materials, to be a guideline of efficient use of the product by providing answers for many common questions. RedSafe has its excitation not only at ultraviolet wave such as UV-A (especially 270nm) and UV-B (300nm), but also at the visible light wave range of 420 ~ 510 nm. RedSafe can be compatible with UV Transilluminator that are generally diffused and also compatible with Blue LED (generally 420nm) known as an alternative for UV recently. RedSafe that drained energy from each wave emits strong green florescence at 520 ~ 550nm (exactly at 535nm) so that it can help DNA analysis after electrophoresis.

Electrophoresis

Electrophoresis is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field. This electro kinetic phenomenon was observed for the first time in 1807 by Ferdinand Frederic Reuss (Moscow State University), who noticed that the application of a constant electric field caused clay particles dispersed in water to migrate. It is ultimately caused by the presence of a charged interface between the particle surface and the surrounding fluid. It is the basis for a number of analytical techniques used in biochemistry for separating molecules by size, charge, or binding affinity.

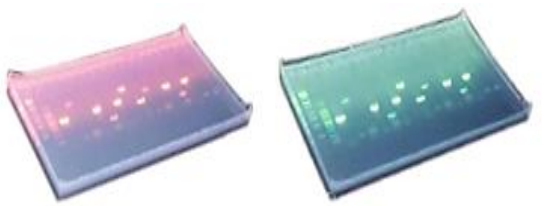
Nucleic acid electrophoresis is an analytical technique used to separate DNA or RNA fragments by size and reactivity. Nucleic acid molecules which are to be analyzed are set upon a viscous medium, the gel, where an electric field induces the nucleic acids to migrate toward the anode, due to the net negative charge of the sugar-phosphate backbone of the nucleic acid chain. The separation of these fragments is accomplished by exploiting the mobility with which different sized molecules are able to pass through the gel. Longer molecules migrate more slowly because they experience more resistance within the gel. Because the size of the molecule affects its mobility, smaller fragments end up nearer to the anode than longer ones in a given period. After some time, the voltage is removed and the fragmentation gradient is analyzed. For larger separations between similar sized fragments, either the voltage or run time can be increased. Extended runs across a low voltage gel yield the most accurate resolution. Voltage is, however, not the sole factor in determining electrophoresis of nucleic acids.

The nucleic acid to be separated can be prepared in several ways before separation by electrophoresis. In the case of large DNA molecules, the DNA is frequently cut into smaller fragments using a DNA restriction endonuclease (or restriction enzyme). In other instances, such as PCR amplified samples, enzymes present in the sample that might affect the separation of the molecules are removed through various means before analysis. Once the nucleic acid is properly prepared, the samples of the nucleic acid solution are placed in the wells of the gel and a voltage is applied across the gel for a specified amount of time.

The DNA fragments of different lengths are visualized using a fluorescent dye specific for DNA, such as ethidium bromide. The gel shows bands corresponding to different nucleic acid molecules populations with different molecular weight. Fragment size is usually reported in "nucleotides", "base pairs" or "kb" (for thousands of base pairs) depending upon whether single- or double-stranded nucleic acid has been separated. Fragment size determination is typically done by comparison to commercially available DNA markers containing linear DNA fragments of known length.

DNA staining

Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. It is commonly abbreviated as "EtBr", which is also an abbreviation for bromoethane. When exposed to ultraviolet light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA. Under the name homidium, it has been commonly used since the 1950s to veterinary medicine to treat trypanosomiasis in cattle, a disease caused by trypanosomes. The high incidence of antibiotic resistance makes this treatment impractical in some areas, where the related isometamidium chloride is used instead. Ethidium bromide may be a mutagen, a carcinogen, or a teratogen, although this depends on the organism exposed and the circumstances of exposure.

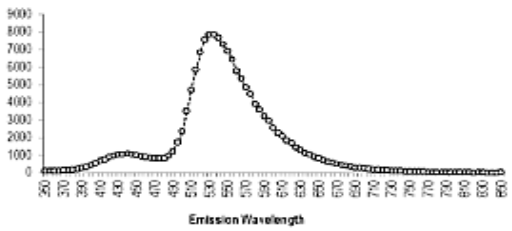


<EtBr with red fluorescence> <RedSafe with green fluorescence>

With that reasons relative to EtBr, iNtRON Biotechnology released a new and safe nucleic acid stain, an alternative to the traditional EtBr stain for detecting nucleic acid in agarose gels. RedSafe has its excitation not only at UV-A or UV-B wavelength which is similar with EtBr but also at 420 ~ 490 nm which is the visible light's wavelength. In accordance with the excitation, it emits green light at 535 nm and is far more sensitive than EtBr. Moreover, it is non-mutagenic and non-cytotoxic, so it is able to perform environmentally safe test.

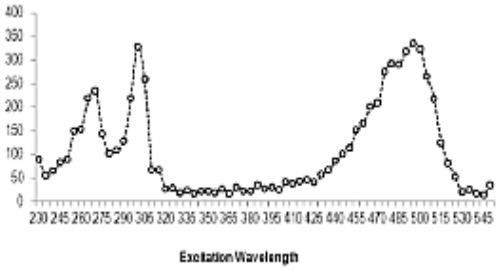
Product description of RedSafe

The analysis of Fluorescence scanning for Emission wavelength of RedSafe demonstrates its results at interval of 5nm from 350 to 850 nm. As a result of this, it showed the centerpeak at 530 ~ 535 nm.



<Fig. 1.> Fluorescence Scanning for Emission Wavelength of RedSafe Nucleic Acid Staining Solution

Fluorescence scanning for its excitation wavelength was performed at interval of 5nm from 230 to 550nm. As a result of this, it demonstrated its peaks at several intervals and its center peaks at 270 nm, 300 nm, 480 nm, 495 nm.



<Fig. 2.> Fluorescence Scanning for Excitation Wavelength of RedSafe Nucleic Acid Staining Solution

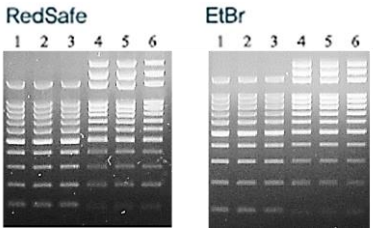
Filters

Image Analyzers using after electrophoresis consist of UV transilluminater, Camera or Analyzing software. A filter that enables to transmit light selectively is mostly able to combine with lens in front of the camera.

Although the basic light may be blue colour, it transmits only the light that each filter allows to transmit. (In case of 520~540 nm, it transmits green light and in case of 590 nm, it transmits orange colour)

-In case of using typical EtBr filter

In case of 590 nm filter optimized to EtBr and general labs are using, fluorescent signal from RedSafe is able to be detected, but not for obtaining the best image.

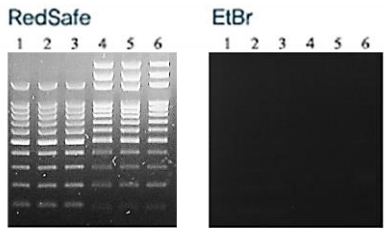


<Fig. 3.> Comparison between RedSafe and EtBr under 590 nm Filter.

In this reason, it can be misunderstood that RedSafe is less sensitive than EtBr if the optimized filter to RedSafe is not used. However, it is able to obtain better images from RedSafe when using an appropriate filter.

- In case of using 520 ~ 540 nm filter

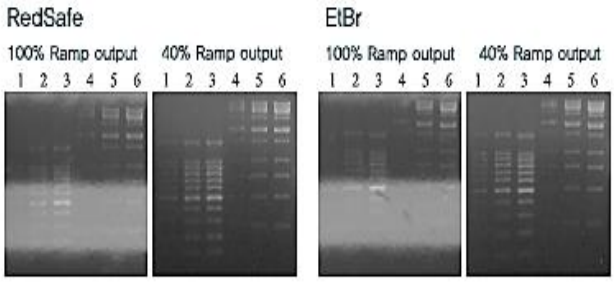
When using 520 ~ 540 filter suitable for RedSafe, RedSafe shows brighter and clearer bands, but EtBr rarely shows bands in the range. Therefore, it is recommended to use 530 or 535nm filter to obtain optimized electrophoretic results.



<Fig. 4.> Comparison of RedSafe with EtBr under 530 nm Filter. Lane 1/2/3, 1/3/5 ul of SiZer-100 DNA Marker respectively; Lane 4/5/6, 1/3/5 ul of SiZer-1000 DNA Marker respectively.

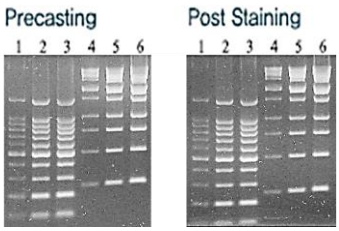
- In case of no filter

When there is no suitable filter for RedSafe, it is able to obtain electrophoretic images. However, The background noise can occur because it may cast the UV ramp behind the gel in case of investigating ultraviolet light which is strong enough to cast the ramp of the UV transilluminator. In this case, good images can be obtained from both RedSafe and EtBr by reducing output of the UV ramp less than 40% and casting it with not enough output to see the ramp.



<Fig. 5.> Comparison of RedSafe with EtBr without filter. Lane 1/2/3, 1/3/5 ul of SiZer-100 DNA Marker respectively; Lane 4/5/6, 1/3/5 ul of SiZer-1000 DNA Marker respectively.

In general, researchers use the precast protocol when preparing agarose gel. However, due to the nature of electrophoresis, RedSafe moves upward(+ charge) and DNA moves downward as time went on. Therefore, the background cannot be stable, and can show its bright top and dark bottom. This is the characteristic of all staining dye including EtBr. Sometimes, few researchers ask for whether Post staining is possible. In conclusion, it is possible. Its protocol is also the same as the protocol using EtBr. If you dilute RedSafe™ 20,000X solution 20,000 fold to make a 1X staining solution in the running buffer and submerge the gel for 10 min., and a very clear image of electrophoresis can be obtained without any background noise. Also, small sizes of fragment can be brightly observed because RedSafe is left on the bottom of the gel evenly.



<Fig. 6.> Comparison of staining methods between Precasting and Post staining / Lane 1/2/3, 1/3/5 ul of SiZer-100 DNA Marker respectively; Lane 4/5/6, 1/3/5 ul of SiZer-1000 DNA Marker respectively.

However, in case of post staining, it requires to establish optimal conditions because the staining speed can vary considerably in the gel's concentration and thickness and the DNA fragment can spread as time went on. Especially because small fragments can spread much more, the establishment of optimal conditions depending on the experimental environment or condition is required. On the other hand, in case of precast protocol, the band can be dark regarding small fragments but it can be comparably sharp because the result is analyzed immediately followed by electrophoresis. one another advantage is to reduce the time expected to be consumed. Therefore, the two staining protocols are not a matter of advantage or disadvantage, but a matter of choice.

Redsafe's toxicity is markedly much lower than EtBr. The Ames test, in vitro mammalian cell chromosome aberration test, acute oral toxicity test and mouse marrow chromophilous erythrocyte micronucleus test performed under 50X RedSafe staining solution have shown that RedSafe is not hazardous. However, the undiluted solution of RedSafe contains solvents such as DMSO, so it requires special care in handling not to get on skin. To touch the gel by naked hand itself is not hazardous, but it is suggested to wear gloves due to safety accident such as short circuit from the tank of electrophorese.

Epilogue

EtBr, the conventional method, is cost-effective in staining DNA & RNA efficiently. However, EtBr is highly mutagenic and has detrimental effects on both the human body and the experimental environment. RedSafe that can replace the conventional method is equivalent to or superior than EtBr and the best experimental results can be obtained on the basis of understandings or right usage of the product.

Both Precasting protocol and post staining protocol are possible with this product and also it is considered that DNA staining by PAGE also is possible.

The best result will be obtained when using 535nm filter, 590nm filter for EtBr can reduce the signal. If there is no filter, a good result can be obtained by decreasing the output of UV ramp itself. Moreover, RedSafe can help the researchers' health and safety, so we expect that RedSafe replaces EtBr and takes its place.

Q & A

Q : What is the shelf life ?
-1 Year at RT 2 years at 4'C

Q : Shipment temperature ?
-Room temperature

Q : Is RedSafe suitable for UV illumination ?
-RedSafe™ is suitable for both UV-A, UV-B illumination.

Q : Does RedSafe™ perform with low concentration of DNA ?
-RedSafe™ can perform with low concentration of DNA. However, the smaller fragments of less than 300bp are not as bright as the bigger one.

Q : Can we use RedSafe for RNA as well as DNA ?
-RedSafe™ can be used for RNA as well as DNA. However, due to mobility shift, please reduce the amount of RNA or follow post staining protocol in case that there is a lot of RNA.

Q : We usually add RedSafe to gel. Is it okay for staining gel after loading ?
-RedSafe is also suitable for Post staining protocol.

Q : The standard filters (wavelength) for EtBr are also suitable for RedSafe™ ?
-It is possible, but not recommended to use. To get the best result, please use the filter ranging in wavelength between 520 ~ 540 nm.

Q : Does RedSafe™ interfere with downstream applications ?
-It is avoidable to leave RedSafe™ in downstream solution. You may purify the DNA solution with purification kit.

Q: How should RedSafe™ be diluted when using post staining protocol?
-Add 5ul of RedSafe™ in 100ml of buffer solution. You may also adjust concentration according to your experiment.

Q: Is RedSafe™ impenetrable to latex gloves ?
-RedSafe™ is impenetrable to latex gloves.

Q : Is it possible to add RedSafe™ when agarose is dissolved in TAE buffer on the hot plate and magnetic stirrer during the heating process ?
-It is not recommended to add RedSafe™ in hot agarose solution. We recommend that after making agarose solution, please cool it by about 60℃ and then add RedSafe™. After that, shake gently and mix well.

Feature

CHARACTERISTICS

- Used for detecting double-strand DNA and single-stranded RNA
- Alternative to the ethidium bromide staining
- As sensitive as EtBr or more sensitive than that
- Non-toxic, non-mutagenic and non-carcinogenic
- No hazard waste

CONTENTS

- RedSafe™ Nucleic Acid Staining Solution (20,000x)
  1 ml

STORAGE CONDITION

- Store at room temperature and stable for more than 12 months. For more stable use, should store at 4℃ (Stable for more than 24 months).

APPLICATION

- Visualization of DNA and RNA bands during agarose gel electrophoresis
- Isolation of DNA fragments for subcloning without introducing mutations normally caused by EtBr.