

Application Note

Ethidium Bromide

Introduction

Ethidium Bromide is an intercalating agent which resembles a DNA base pair. Due to its unique structure, it can easily intercalate into DNA strand. Therefore, it is commonly used as nucleic acid fluorescent tag in various techniques of the life science field. The Ethidium bromide is being used since the 1950's, when used in veterinary treat for cattle. It is common knowledge the EtBr may act as a strong mutagen, while it's carcinogenic and teratogen aspects still have to be well clarified. Following its toxicity, it is defined as harmful by all routes of entry; inhalation, ingestion, or skin absorption. The EtBr causes eye, skin, mucous membranes and upper respiratory tract irritation.

Similar to most of the fluorescent compounds, the EtBr is an aromatic molecule. Its core can be defined as phenanthridine

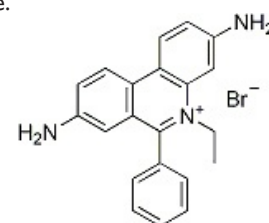
– an isomer of acridine.

The reason for Ethidium Bromide's intense fluorescence after binding with DNA is the hydrophobic environment found between the base pairs. By moving into this environment and away from the solvent, the EtBr cation is forced to shed any water associated molecules. As water is a highly efficient fluorescent quencher, the removal of water molecules allows the EtBr to fluoresce.

The EtBr common dye has several biological applications. Regarding DNA it is usually used for double strand from PCR, restriction digest etc. ssRNA can also be detected, since it usually folds back onto itself and consequently provides local base pairing for the dye to intercalate.

General Information:

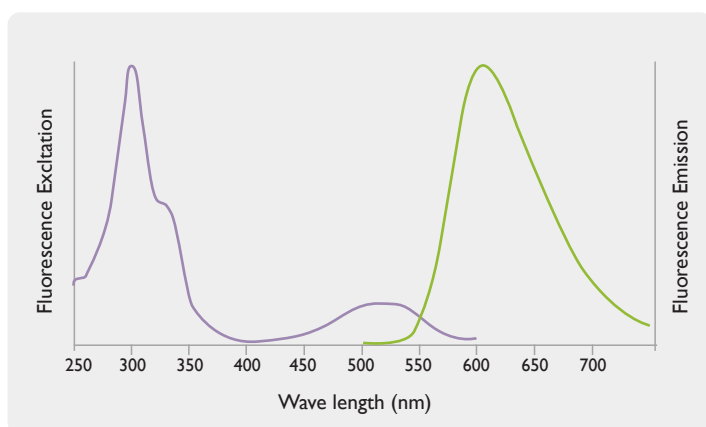
Chemical Name:	Ethidium Bromide
Chemical Formula:	$C_{21}H_{20}N_3Br$
Molecular Weight:	394.4
Synonyms:	Ethbromide; Dromilac; Homidium Bromide; EtBr; RD 1572 and more.



Ethidium Bromide structure

Detection

The Ethidium Bromide can be detected in most kinds of the biological experiment while using its excitation spectrum peaks (300nm and 520nm) and the emission peak at 600nm.



Ethidium Bromide excitation and emission spectrums (Accordingly in blue and red)
Image courtesy of Invitrogen

DNR Laboratories Detection Tip

The EtBr dye can be easily detected by the MicroBIS with the 470nm excitation source and the standard orange filter.

There are several configurations which can be used to detect this dye, and the user must choose the ultimate parameters for his/her experiments, according to his experiment's specification (camera settings and illumination).

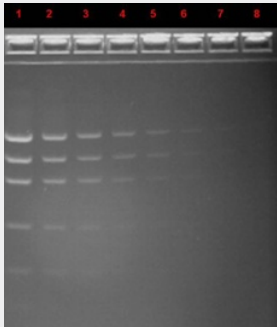
General parameters for EtBr dye detection in the MicroBIS:

Parameter	Value
Exposure Time	1.2 Seconds
Gain	1
Brightness	0
Scan Mode	Fast
Illumination	470nm
Filter	Orange (standard filter)

DNR EtBr Image

General Information:

Bio-Imaging System: MicroBIS
 Gel: E-Gel® 2% with SYBR Safe™, Catalog number: G6000-02, Invitrogen
 Sample: Low DNA Mass Ladder™ Catalog number 10068-013, Invitrogen.



EtBr detection by the MicroBIS
 Electrophoresis was carried out with Low DNA Mass Ladder™.

Lanes concentration:

Lane	Concentration					
1	200ng	120ng	80ng	40ng	20ng	10ng
2	100ng	60ng	40ng	20ng	10ng	5ng
3	50ng	30ng	20ng	10ng	5ng	2.5ng
4	25ng	15ng	10ng	5ng	2.5ng	1.25ng
5	12.5ng	7.5ng	5ng	2.5ng	1.25ng	0.62ng
6	6.25ng	3.75ng	2.5ng	1.25ng	0.62ng	0.31ng
7	3.12ng	1.87ng	1.25ng	0.62ng	0.31ng	0.15ng
8	1.56ng	0.93ng	0.62ng	0.31ng	0.15ng	0.07ng

The above image shows that the MicroBIS minimum detection with EtBr is 1.25ng. By using the MicroBIS UV light table the minimum detection increase in one fold (down to pg).

Bibliography

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