

Application Note

OVERVIEW of COMMON FLUORESCENT dyes USED in NUCLEIC ACID QUANTITATION

INTRODUCTION

Quantitation of DNA in solution is an extremely common application in all areas of molecular biology research. Knowing the amount of DNA in a sample is an important first step to such applications as:

- DNA sequencing
- cDNA library development
- Purification of DNA fragments for subcloning
- Identification of contaminating DNA in recombinant protein products

While many researchers use absorbance readings at 260nm for DNA quantitation, there are serious limits on the sensitivity and accuracy of this technique. Contamination from proteins, carbohydrates, ssDNA, RNA, and free nucleotides often make the results of 260nm reading a "best guess".

Although the use of fluorescent dyes for DNA quantitation is more expensive, the benefits often outweigh the cost disadvantage. Fluorescent detection of DNA is roughly 100 to 1000 times more sensitive than an absorbance reading. Also, there are several dyes that are specific for dsDNA, ssDNA, and RNA. Fluorescent dyes in general are not affected by the presence of common contaminants such as protein and carbohydrate molecules.

This document will outline many of the common fluorescent dyes used to detect and quantify DNA. Dyes covered in this paper are DAPI, Hoechst Dyes, PicoGreen, RiboGreen, OliGreen, and cyanine dyes such as YO-YO, ethidium bromide, and SybrGreen.

Much of this information is a summary of the material covered in Sections 8.1 and 8.3 of the Handbook of Fluorescence Probes and Research Products from Molecular Probes (www.probes.com/handbook). Additional information can be found there or in the sources listed in the bibliography at the end of the text.



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OVERVIEW of Dyes

Below is a table outlining the basic properties of common dyes used for quantitation of nucleic acids. Details on the dyes can be found in separate sections of this paper.

Name of Dye	Ex/Em (in nm)	Specificity	Sensitivity	Factors Affecting Performance	Advantages	Disadvantages
DAPI	360/465	A-T		Detergents, polyphosphates, dextran sulfate	inexpensive,	strongly A-T selective
Hoechst 33258	360/465	A-T of dsDNA	10 ng/mL	pH, SDS	inexpensive, sensitive	strongly A-T selective
PicoGreen	485/535	all dsDNA	250 pg/mL	none demonstrated	simple, sensitive, no reaction cleanup necessary	cost
RiboGreen	485/535	G of all nucleic acids	200pg/mL	presence of any DNA	quantitation of RNA with simple DNase treatment	
OliGreen	485/535	T of all nucleic acids	100 pg/mL	presence of dsDNA or RNA	quantitation of ssDNA, sensitivity	will detect dsDNA and RNA present
cyanine dyes (e.g. YO-YO-1)	varies	dsDNA, some single-stranded oligonucleotides	0.5 to 2.5 ng/mL	salt, ethanol, SDS; some variants show base selectivity	inexpensive, relatively sensitive	less sensitive or selective than PicoGreen
ethidium bromide	520/605	dsDNA and RNA	2 ng (per band, agarose gel)	degrades in the presence of sodium nitrate and hypophosphorous acid, must be stored in the absence of light	very inexpensive, good sensitivity	toxic mutagen with possible carcinogenic properties as well, not used with microplate readers
SybrGreen I	485/535	dsDNA	25 pg (per band, agarose gel)	must be stored in the absence of light	inexpensive dye for use in qPCR reactions, not mutagenic	not commonly used with microplate readers

DAPI

The chemical 4', 6-diamidino-2-phenylindole is usually referred to as DAPI. This is a fluorescent probe that shows a high specificity for DNA. It falls into the class of "minor-groove binders", in that it only forms a fluorescent complex when the molecule is bound to the minor groove of A-T rich sequences of DNA. It does form intercalative complexes with DNA in other regions, such as GC sequences or ssDNA, however these complexes are generally non-fluorescent.

DAPI has been used as a DNA-specific probe in a number of applications, including flow cytometry, chromosome staining, and DNA visualization and quantitation in histochemistry and biochemistry.

Although other minor-groove binding dyes (such as the Hoechst dyes) show more overall fluorescence yield than DAPI, it still shows a nearly 20-fold increase in fluorescence when bound to dsDNA. DAPI emits light in the blue wavelengths, generally around 460nm. Fluorescence of DAPI also greatly increases in the presence of detergents, dextran sulfate, polyphosphates, and polyanions.



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Hoechst Dyes

Hoechst dyes were originally developed by the German company Hoechst AG, which started out as a synthetic clothing dye manufacturer in the 1880's. The company added the manufacture of chemicals in the early twentieth century. The dyes are currently available from a number of different fluorescent dye distributors today.

These dyes, which are also referred to as bisbenzimidazole dyes, are similar to DAPI in that they are minor-groove binders to DNA. While these dyes also show A-T selectivity, they also have much more complex DNA affinities. These dyes are generally excited near the UV range of light (about 360nm), and emit light in the blue range at around 460nm. This makes for a fairly large Stokes shift. Therefore, these dyes are particularly useful for multiplex labeling.

The most common variation for DNA detection and quantitation is Hoechst 33258. This dye shows a strong selectivity for dsDNA, but does not show a significant increase in fluorescence when in the presence of proteins. Sodium dodecyl sulfate (SDS) will cause a significant increase of fluorescence in the Hoechst 33258 dye. Another factor in fluorescence yield is pH. A pH of 5 will give a much higher fluorescent yield than will a pH of 8. Hoechst 33258 can be used to quantitate DNA down to 10ng/mL.

Another variant for DNA quantitation is Hoechst 33342. This dye is slightly more cell-membrane permeable than Hoechst 33258 and is commonly used in apoptosis studies.

PicoGREEN

PicoGreen is a fluorescent dye developed and patented by Molecular Probes. This widely used dye shows a very strong increase in fluorescence (>1000 times) in the presence of dsDNA, and does not show a significant increase in fluorescence in the presence of proteins, carbohydrates, ssDNA, RNA, or free nucleotides. This makes it possible to quantitate DNA without purification after PCR amplification. Also, the dye does not show any A-T or G-C selectivity, so it can easily be used on DNA from any source. PicoGreen has been shown to have a sensitivity of detecting DNA down to 250 pg/mL. Overall assay linearity has been shown to extend through four orders of magnitude.

RiboGREEN

RiboGreen can be used to detect RNA due to its large increase in fluorescent yield upon binding to nucleic acids. RiboGreen will also bind and fluoresce in the presence of DNA (both single- and double-stranded). However, a simple treatment of the sample with DNase will allow for sensitive quantitation of the remaining RNA. Using two dye concentrations, three orders of magnitude of sensitivity are possible, starting at 1 ng/mL. RiboGreen shows some base selectivity, with a 60% decrease in fluorescence in the presence of poly(G) fragments and virtually no fluorescence in poly(U) or poly(C) fragments.



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OliGREEN

OliGreen can detect as little as 100 pg/mL of ssDNA, and is therefore up to 500 times more sensitive than using ethidium bromide in electrophoretic gels. It does, however, show significant base selectivity for thymine, with little or no selectivity shown for adenine, cytosine, or guanine. OliGreen also exhibits significant increase in fluorescence in the presence of dsDNA and RNA.

CYANINE DYES

This is a class of fluorescent dyes for nucleic acids that includes the TOTO and YOYO families of dyes (referred to as dimeric cyanine dyes), ethidium bromide, and Sybr Green.

Dimeric cyanine dyes can be used to quantitate dsDNA, ssDNA, and RNA in solution due to the low intrinsic fluorescence in the absence of nucleic acids and the high levels of fluorescence yield upon nucleic acid binding. They are not as sensitive as the PicoGreen family of fluorescent dyes, as they only show a linear range of two orders of magnitude with an ultimate sensitivity of 0.5 ng/mL. Some applications of these dyes include:

YOYO-1 for measuring DNase activity, quantitating oligonucleotides and PCR products
YO-PRO-1 for quantitating dsDNA in solution (reported sensitivity of 2.5 ng/mL)
TOTO-1 for quantitating PCR amplification products
PO-PRO-3 for quantitating DNA

Additional cyanine dyes and their applications include:

Ethidium bromide for quantitating DNA yield from PCR (in conjunction with an agarose gel) and SybrGreen for quantitating nucleic acids on plastic wrap or paraffin. This dye is also commonly used in real time PCR (qPCR) reactions for DNA quantitation. However, neither of these dyes are commonly used in microplate readers.

Bibliography

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