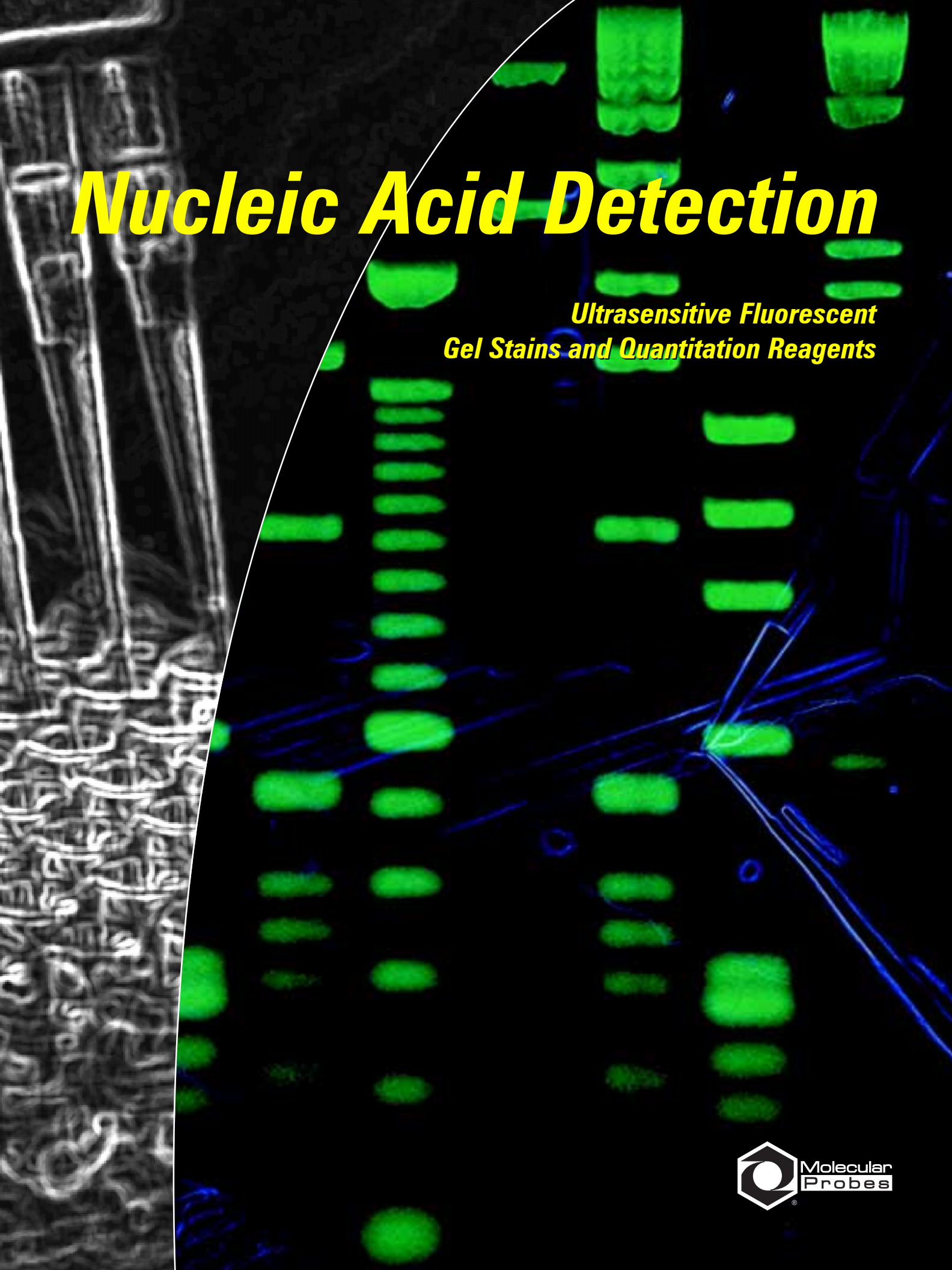


Nucleic Acid Detection

Ultrasensitive Fluorescent Gel Stains and Quantitation Reagents

Molecular Probes®

Bright Signal *Low Background* High Sensitivity

Molecular Probes has developed nucleic acid stains that, in addition to having high affinities for nucleic acids, also exhibit very high fluorescence enhancements upon binding (>300-fold) compared to conventional stains such as ethidium bromide and Hoechst 33258. Our dyes also have high extinction coefficients and quantum yields, resulting in extremely strong fluorescence signals. All of these properties combine to make our nucleic acid stains the easiest to use, most reliable and highest-sensitivity dyes for gel staining and solution quantitation.

SYBR dyes: the most sensitive gel stains

SYBR nucleic acid gel stains are the most sensitive stains available for nucleic acid detection in gels, allowing you to use a fluorescent dye to visualize bands that previously could only be detected using labor-intensive silver staining or radioactive labeling techniques.

Advantages

Highly sensitive. Up to 25-fold more sensitive than ethidium bromide, SYBR dyes provide sensitivity rivaling that of silver staining.

Easy to use. One-step staining and detection does not require destaining or washing.

Compatible with molecular biology techniques. Stained nucleic acids can be used for Northern or Southern blotting and in enzymatic reactions such as ligations, restriction digests, amplification reactions and *in vitro* transcription. If desired, the stain can be removed from extracted bands by a simple ethanol precipitation.

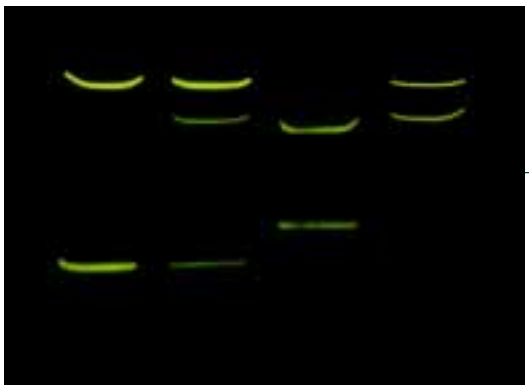


Figure 1. *Direct visualization of SSCP in exon 1 of human K-ras using SYBR Gold nucleic acid gel stain. Lane 1 contains wild-type DNA and lanes 2–4 contain DNA from various adenocarcinoma samples with mutant alleles. Image contributed by Valerie DeGroff and Chris Weghorst, Ohio State University.*

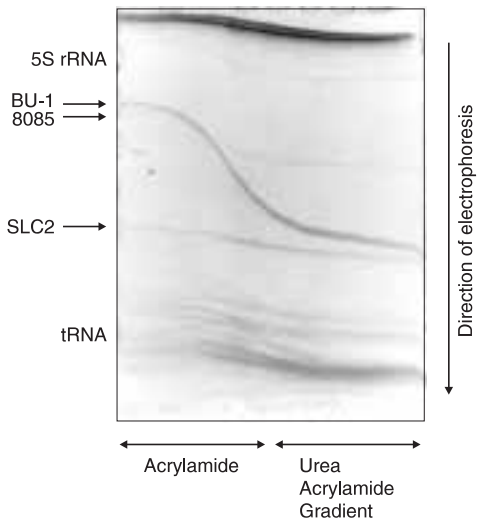


Figure 2. *Negative image of a DGGE gel stained with SYBR Green II dye. The different migration patterns of 5S rRNA from Leptospirillum ferroxidans BU-1 strain, Thiobacillus thiooxidans ATCC 8085 strain and an iron-oxidizing heterotrophic bacterium, SLC2, can be seen in the left half of the gel. Image contributed by Daphne Stoner, Idaho National Engineering Laboratory.*

SYBR Gold nucleic acid gel stain

The most sensitive and versatile fluorescent stain for use with UV transilluminators

This newest SYBR dye outperforms ethidium bromide in any gel system, including agarose and polyacrylamide gels, native gels, formaldehyde gels, glyoxal gels and urea gels.[1] The stain penetrates thick gels easily for fast and even staining. It is the most sensitive stain for dsDNA, ssDNA and RNA using a standard 300 nm UV transilluminator, enabling you to obtain high sensitivity without using expensive laser scanners.

Applications

Northern blotting

Stained RNA transfers easily onto nitrocellulose or nylon membranes by standard blotting methods, without loss of the sample. The stain washes off of the RNA during the prehybridization step and does not interfere with hybridization.

SSCP analysis

SYBR Gold stain is ideal for "cold" single-strand conformation polymorphism (SSCP) analysis, eliminating the need for radioactivity in this gel-based allele detection assay (Figure 1).

PCR-based assays

As sensitive as silver stains, but much easier to use,[1] SYBR Gold stain provides the ideal detection method for PCR-based gel assays requiring high sensitivity, such as the telomeric repeat amplification protocol[2] (TRAP).

SYBR Green II nucleic acid gel stain

A high-sensitivity dye designed for staining RNA in gels

SYBR Green II stain shows especially good sensitivity for RNA, while also staining dsDNA and ssDNA. The ideal dye for use with laser scanning instruments, SYBR Green II stain exhibits very low background fluorescence in the gel and has spectral characteristics that match common light sources and filter sets.

Applications

Northern blotting

SYBR Green II stain shows high-sensitivity RNA staining in formaldehyde gels without the need for destaining. Staining with SYBR Green II dye does not interfere with subsequent RNA transfer or blotting.[3] Staining the gel prior to blotting provides a means of normalizing the hybridization signals.[4]

DGGE analysis

This stain makes it possible to perform a very sensitive assay that characterizes species in a mixed microbial population. The assay is based on the migration of bacterial 5S rRNA during denaturing gradient gel electrophoresis[5] (DGGE, Figure 2).

SSCP analysis

SYBR Green II stain provides a simple method for high-sensitivity, nonradioactive single-strand conformation polymorphism (SSCP) analysis.[6–8]

References

1. Anal Biochem 268, 278 (1999); **2.** Mol Pathol 51, 342 (1998); **3.** J Chinese Biochem Soc 32, 1 (1995); **4.** BioTechniques 26, 46 (1999); **5.** Appl Environ Microbiol 62, 1969 (1996); **6.** Diagnostic Mol Pathol 5, 260 (1996); **7.** Anal Biochem 236, 373 (1996); **8.** Proc Natl Acad Sci USA 94, 10745 (1997).

SYBR Green I nucleic acid gel stain

A dsDNA-selective dye with exceptionally low background

The well-established SYBR Green I stain preferentially stains dsDNA, making it especially useful for assays where the presence of contaminating RNA or ssDNA might otherwise obscure the results. With exceptionally low background fluorescence and spectral characteristics that closely match light sources and filter sets in existing instruments, SYBR Green I stain is ideal for use with laser scanners.

Applications

Complex samples

Preferential dsDNA staining makes it easy to detect dsDNA patterns, such as apoptosis ladders, even in crude extracts (Figure 3).

DNA typing

SYBR Green I stain shows much higher DNA staining sensitivity than does ethidium bromide and can replace silver staining or radioisotope labeling in gel-based DNA-typing assays.[1–6]

PCR-based assays

The stain also improves the sensitivity of other gel-based PCR assays, such as viral detection assays [7] and the telomeric repeat amplification protocol [8] (TRAP). Increased sensitivity means improved accuracy in competitive reverse transcription-PCR (RT-PCR) because fewer cycles are required.[9–11]

Band-shift assays

SYBR Green I stain can replace radioactivity for detection of protein-bound and unbound DNA in band-shift assays [12,13] (Figure 4).

DNA damage assays

SYBR Green I stain makes assays for DNA damage easier, safer and more sensitive. It has been used to replace tritium-labeling of DNA in a pulsed field gel electrophoresis (PFGE) assay [14] and to increase the sensitivity of the popular comet assay.[15]

References

1. BioTechniques 19, 223 (1995); **2.** BioTechniques 22, 976 (1997); **3.** Nature Biotech 16, 91 (1998); **4.** Biochim Biophys Acta 1360, 193 (1997); **5.** Mol Cell Probes 9, 145 (1995); **6.** J Forensic Sci 44, 87 (1999); **7.** J Virol Meth 55, 153 (1995); **8.** J Biol Chem 274, 7264 (1999); **9.** J Biol Chem 274, 21893 (1999); **10.** PCR Meth Appl 4, 234 (1995); **11.** BioTechniques 22, 1107 (1997); **12.** FASEB J 10, A1128, abstract #751 (1996); **13.** J Biol Chem 274, 27287 (1999); **14.** Nucleic Acids Res 25, 2945 (1997); **15.** www.kineticimaging.com/komet.htm.

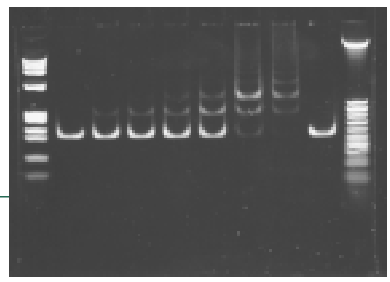


Figure 4. *Band-shifts detected with SYBR Green I stain. Samples containing 50 ng of a 208 bp DNA fragment and varying amounts of a mutant enzyme (EcoRI/Gln 111) were electrophoresed through a native polyacrylamide gel then stained with SYBR Green I stain. Lanes 1 and 10 contain size markers; lanes 2 through 9 contain 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 0 μM EcoRI/Gln 111.*

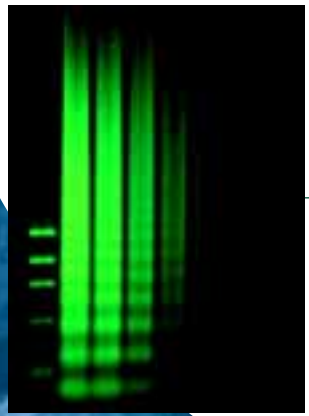


Figure 3. *Detection of DNA fragments in apoptotic cells. DNA extracts from HL-60 cells treated with the apoptosis-inducing compound camptothecin were separated on an agarose gel then stained with SYBR Green I nucleic acid gel stain. The 200 to 5000 bp DNA fragments characteristic of apoptotic cells appear as "ladders." Cell preparations were gifts of Zbigniew Darzynkiewicz, Cancer Research Institute, New York Medical College.*

SYBR Green/Gold photographic filter

Documenting a fluorescent image on film or with a CCD camera allows the image to be integrated over time, so you will see fluorescence signals not detectable with the human eye alone. To obtain the highest sensitivity with the SYBR nucleic acid gel stains using a UV transilluminator and Polaroid black-and-white print film, we recommend the use of the SYBR Green/Gold photographic filter. This simple and inexpensive 3 × 3–inch gelatin filter blocks out background UV light while allowing the maximum amount of SYBR stain fluorescent signal to reach the camera. For CCD cameras, laser scanners or cameras requiring screw-in glass filters, please contact the instrument manufacturer for the appropriate filters.

PicoGreen, RiboGreen and OliGreen dyes: the most sensitive solution quantitation reagents

Molecular Probes' PicoGreen, RiboGreen and OliGreen quantitation reagents show very high fluorescence enhancements upon binding to nucleic acids. This characteristic provides a simple, high-sensitivity method for quantitating nucleic acids in solution. The high sensitivity means you save more of your precious samples for research. The one-step assays are easily adaptable for use in high-throughput settings.

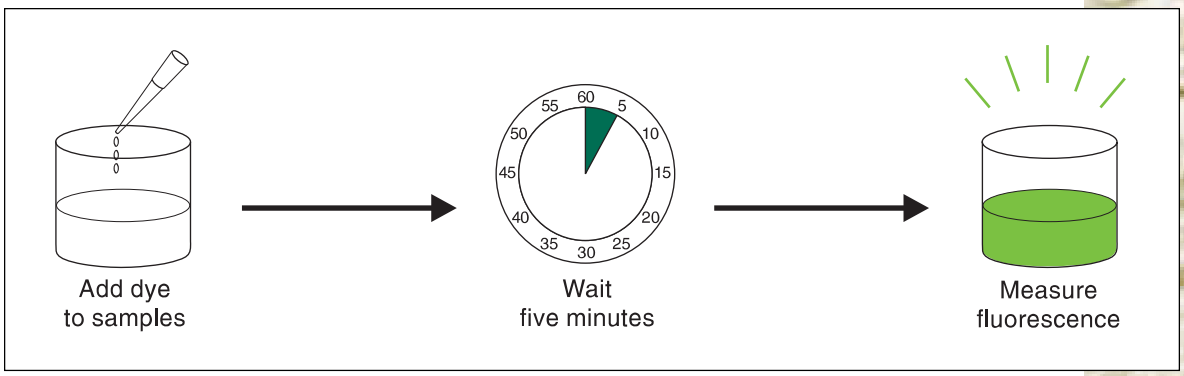
Advantages

Sensitive and accurate. These fluorescent dyes are orders-of-magnitude more sensitive than UV absorbance (A_{260}) readings or assays using Hoechst 33258. And in contrast to A_{260} measurements, nucleic acids can be quantitated without interference from proteins or free nucleotides.

Fast and easy. These one-step assays require only a five-minute incubation and are easily adapted to robotic high-throughput quantitation.

Compatible with most instruments. Fluorescent signals match the excitation sources and optical filters used for fluorescein, features commonly available with most fluorescence microplate readers and fluorometers.

Molecular Probes' solution quantitation assays. *Ideal for a single sample or in high-throughput applications, these five-minute assays require just a simple fluorometer or fluorescence microplate reader to provide sensitivity that is orders-of-magnitude greater than UV absorbance (A_{260}) readings.*



PicoGreen dsDNA quantitation reagent

The most sensitive dye for solution quantitation of dsDNA

Using the PicoGreen reagent, you can selectively detect as little as 25 pg/mL of dsDNA (Figure 5) in the presence of ssDNA, RNA and free nucleotides.[1] The assay is linear over three orders of magnitude and has little sequence dependence, allowing you to accurately measure DNA from many sources, including genomic DNA, viral DNA, miniprep DNA or PCR amplification products.

Applications

PCR-based assays

The PicoGreen assay makes it possible to design simplified assays for genotyping and other PCR-based techniques.[2] You can also accurately measure yields from PCR, RT-PCR, STR or cycle sequencing reactions prior to gel electrophoresis[3–6] or labeling reactions.[7]

DNA damage assays

You can take advantage of the PicoGreen assay's dsDNA selectivity to design rapid, sensitive and quantitative DNA-damage assays based on denaturation measurements. Because the assay preferentially detects dsDNA, the fluorescence signal decreases as the dsDNA is denatured.[8–10]

Enzyme activity assays

The PicoGreen reagent makes it easy to perform high-throughput microplate assays to measure telomerase, reverse transcriptase or DNA polymerase activity.[11,12]

Genomic DNA

PicoGreen reagent allows you to quantitate genomic DNA isolated from blood, buccal scrapes, tissues or cultured cells to enable accurate genotyping analysis, while only using a small amount of sample.[13]

Complex mixtures

You can quantitate dsDNA in restriction digests, lipid–DNA complexes,[14] pharmaceutical or recombinant protein preparations and environmental samples.[15]

Viral DNA

The PicoGreen reagent provides a fast and reproducible method for quantitating viral DNA, while providing higher sensitivity and using less sample than conventional absorbance methods.[16]

References

1. Anal Biochem 249, 228 (1997); **2.** BioTechniques 24, 206 (1998); **3.** BioTechniques 20, 676 (1996); **4.** Anal Biochem 246, 140 (1997); **5.** Nucleic Acids Res 24, 2623 (1996); **6.** BioTechniques 21, 372 (1996); **7.** Nature Biotech 17, 798 (1999); **8.** Anal Biochem 270, 195 (1999); **9.** Anal Chem 71, 4423 (1999); **10.** Cell Mol Biol 45, 211 (1999); **11.** BioTechniques 21, 664 (1996); **12.** Proc Natl Acad Sci USA 93, 6091 (1996); **13.** BioTechniques 23, 18 (1997); **14.** Human Gene Ther 9, 341 (1998); **15.** BioTechniques 23, 532 (1997); **16.** Anal Biochem 274, 283 (1999).

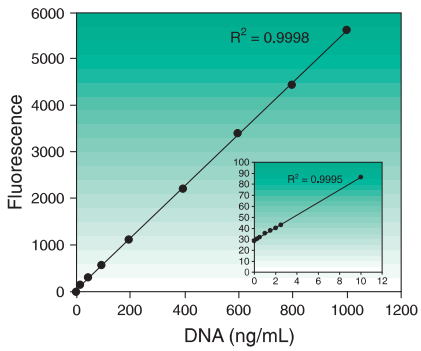


Figure 5. *Measurement range and sensitivity of the PicoGreen dsDNA quantitation assay. Calf thymus DNA was incubated with the PicoGreen reagent for five minutes. Fluorescence measurements were made using a fluorescence microplate reader with excitation at 485 +/– 4.5 nm and emission detection at 525 +/– 4.5 nm. Fluorescence emission intensity was then plotted as a function of DNA concentration. The inset shows the results from the lower range of the assay.*

RiboGreen RNA quantitation reagent

The most sensitive dye for solution quantitation of RNA

Using the RiboGreen reagent, you can detect as little as 1 ng/mL of RNA [1] (Figure 6). In contrast to UV absorbance measurements (A_{260}), where proteins and free ribonucleotides in the mixture interfere with accurate quantitation, the RiboGreen reagent only measures polymeric nucleic acids. Addition of a DNase digestion step easily converts the procedure into an RNA-selective assay.

Applications

RNA expression analysis

RiboGreen reagent allows you to quantitate the amount of intact RNA in the sample before using it for Northern blotting, S1 nuclease or RNase protection experiments. Using only small portions of your samples, you can obtain reliable RNA quantitation, making subsequent quantitation of specific RNA species more accurate.

Reverse transcription reactions

You can use the RiboGreen assay to quantitate the amount of intact RNA in your sample before setting up reverse transcription reactions for microarray analysis, cDNA libraries, RT-PCR or differential display PCR.

OliGreen ssDNA quantitation reagent

The most sensitive dye for solution quantitation of oligonucleotides

Using the OliGreen reagent, you can detect as little as 100 pg/mL of ssDNA, giving you 1000 times more sensitivity than UV absorbance measurements (A_{260}).

Applications

Primers

OliGreen reagent makes it easy to measure yields from oligonucleotide synthesis, labeling and purification procedures. You can use OliGreen reagent to quantitate PCR primers, RT primers or hybridization probes.

Complex mixtures

You can use OliGreen reagent to quantitate standard, phosphodiester or phosphorothioate oligonucleotides in complex mixtures, including blood, plasma or serum.[2,3]

ssDNA

Using OliGreen reagent, you can quantitate preparations of single-stranded phage or denatured genomic DNA.

References

1. Anal Biochem 265, 368 (1998); **2.** Antisense Nucleic Acid Drug Devel 7, 133 (1997); **3.** Anal Chem 69, 3218 (1997).

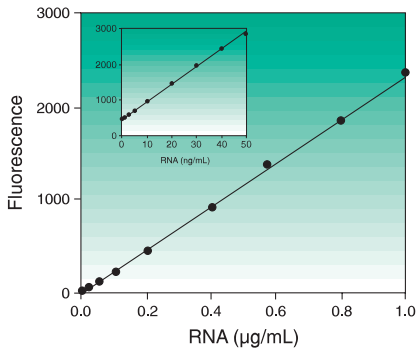


Figure 6. *Measurement range and sensitivity of the RiboGreen RNA quantitation assay. E. coli ribosomal RNA was incubated with a 2000-fold dilution of the dye for the low range assay (inset) or with a 200-fold dilution of the dye for the high range assay (large graph) for five minutes. Fluorescence measurements were made using a fluorescence microplate reader with excitation at 485 +/– 10 nm and emission detection at 530 +/– 12.5 nm. Fluorescence emission intensity was then plotted as a function of DNA concentration.*

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