Fluorescent Dyes and Assays for Genomics and Proteomics Research

GelRed™ & GelGreen™: Safe nucleic acid gel stains ... pp. 2-3
EvaGreen® dye for qPCR ... pp. 4-5
Cheetah™ Taq: Fast activating hot-start Taq ... pp. 4-5
HotStart & WarmStart™ Polymerase Modification Kits ... p. 6
EvaEZ™ DNA Polymerase Activity Assay ... p. 6
PMA™ for bacterial viability ... p. 7
PMA-Lite™ Photolysis Device ... p. 7
AccuLite™ handheld mini-fluorometers ... p. 8
AccuBlue™ and AccuClear™ DNA Quantitation Kits ... p. 8-9
Lumitein™ Protein Gel Stain ... p. 10
AccuOrange™ Protein Quantitation Assay ... p. 11
GelRed™ and GelGreen™ are next-generation fluorescent nucleic acid gel stains designed to replace the highly toxic ethidium bromide (EtBr). Developed by scientists at Biotium, GelRed™ and GelGreen™ are superior to EtBr and other EtBr alternatives by having a combination of low toxicity, high sensitivity and exceptional stability.

EtBr has been the predominant dye used for nucleic acid gel staining for decades because of its low price and generally sufficient sensitivity. However, EtBr has been the predominant dye used for nucleic acid gel staining for decades because of its low price and generally sufficient sensitivity. However, EtBr is a highly mutagenic chemical. The safety hazard and costs associated with decontamination and waste disposal can ultimately make the dye expensive and inconvenient to use. For this reason, alternative gel stains, such as SYBR® dyes, have become commercially available in recent years. While these alternative dyes have reduced mutagenicity, they sacrifice sensitivity and stability. For example, SYBR® Safe has very limited sensitivity while SYBR® Green and SYBR® Gold are much less stable than EtBr. SYBR® dyes also enter cells rapidly to stain mitochondria and nuclear DNA, making it more likely for the dyes to be harmful to cells. Indeed, SYBR® Green I has been shown to strongly potentiate DNA mutation caused by UV light and other mutagens (Ohta, et al. 2001).

Safer options for gel staining

To make safer gel stains, scientists at Biotium used a novel yet very simple concept: reducing genotoxicity by preventing the dyes from entering living cells. We believe that the mutagenicity of a DNA-binding dye can greatly reduced by denying it access to genomic DNA in living cells. Thus, we engineered the chemical structures of GelRed™ and GelGreen™ such that the dyes are incapable of crossing cell membranes. Ames tests have confirmed that GelRed™ and GelGreen™ are nonmutagenic at concentrations well above the concentrations used for gel staining. Furthermore, environmental safety tests showed that GelRed™ and GelGreen™ are non-toxic to aquatic life. Because of this, GelRed™ and GelGreen™ are classified as non-hazardous waste, and can be disposed of regular trash or down the drain. For more information, please download the GelRed™/GelGreen™ Safety Report at www.biotium.com.

Superior sensitivity

GelRed™ and GelGreen™ are highly sensitive for precast or post-electrophoresis gel staining. Designed primarily for use with a 312/302 nm UV transilluminator, GelRed™ is much more sensitive than EtBr. GelGreen™ is compatible with visible blue light transilluminators and UV to blue light converter plates, which protect users and DNA samples from UV light exposure. Using blue light excitation instead of UV when excising DNA from preparative gels greatly improves downstream cloning efficiency (Gründemann and Schömig 1996). GelGreen™ is spectrally similar to SYBR Safe®, but is far more sensitive than the latter.

Another advantage of GelRed™ and GelGreen™ is their chemical stability. You can handle the two dyes the same way as EtBr. This means that the dyes are perfectly stable in water at room temperature for long-term storage, and they can be added to molten agarose for making precast gels. Both dyes are also very photostable and can be handled under normal room light.

Biotium gel extraction kits and DNA ladders are optimized for use with GelRed and GelGreen precast gels. GelRed™ and GelGreen™ also are compatible with popular gel extraction kits and DNA ladders from other suppliers.

References

Search GelRed Biotium or GelGreen Biotium on Google Scholar: GelRed has been cited in more than 1500 publications, and GelGreen has been cited in more than 100 publications to date.


GelRed, GelGreen, and their uses are covered by US patent numbers 7,960,498 and 7,803943. SYBR is registered trademark of Molecular Probes Corp.

1 kb and 100 bp DNA ladders

- Ready-to-use at optimal concentration for loading GelRed™ and GelGreen™ precast gels
  - 1 kb ladder: 250 bp-10 kb
  - 100 bp ladder: 100 bp-1.5 kb
- Also available with loading buffer supplied separately.
Safer, ultra-sensitive alternatives to EtBr and SYBR® dyes

Figure 3. GelRed™ and GelGreen™ gel stains are safer because they cannot penetrate cell membranes to bind DNA in living cells. HeLa cells were incubated at 37°C with 1X SYBR® Safe, GelGreen™ or GelRed™, respectively. Images were taken following incubation with dye for 30 min. The top row shows phase contrast images of the field of cells, the bottom row shows green fluorescence for SYBR® Safe and GelGreen™ and red fluorescence for GelRed™. SYBR® Safe rapidly entered cells and stained nuclei. GelRed™ and GelGreen™ were unable to cross cell membranes, demonstrated by the absence of fluorescence staining.

Table 1. Ordering Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
<th>Unit Size</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>GelRed™ 10,000X in water</td>
<td>41003-T</td>
<td>25 uL</td>
<td>Newer, safer formulation in water (recommended for new users)</td>
</tr>
<tr>
<td></td>
<td>41003</td>
<td>0.5 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41003-1</td>
<td>10 mL</td>
<td></td>
</tr>
<tr>
<td>GelRed™ 10,000X in DMSO</td>
<td>41002</td>
<td>0.5 mL</td>
<td>Original formulation in DMSO</td>
</tr>
<tr>
<td>GelRed™ 3X in water</td>
<td>41001</td>
<td>4 L</td>
<td>Ready to use for post-staining</td>
</tr>
<tr>
<td>GelGreen™ 10,000X in water</td>
<td>41005-T</td>
<td>25 uL</td>
<td>Newer, safer formulation in water (recommended for new users)</td>
</tr>
<tr>
<td></td>
<td>41005</td>
<td>0.5 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41005-1</td>
<td>10 mL</td>
<td></td>
</tr>
<tr>
<td>GelGreen™ 10,000X in DMSO</td>
<td>41004</td>
<td>0.5 mL</td>
<td>Original formulation in DMSO</td>
</tr>
<tr>
<td>Ready-To-Use 1 kb DNA Ladder</td>
<td>31022</td>
<td>150 lanes</td>
<td>Optimal concentration for GelRed™/GelGreen™ precast gels*</td>
</tr>
<tr>
<td>Ready-To-Use 100 bp DNA Ladder</td>
<td>31032</td>
<td>150 lanes</td>
<td></td>
</tr>
<tr>
<td>1 kb DNA Ladder</td>
<td>31021</td>
<td>30 ug</td>
<td>Stand-alone ladders</td>
</tr>
<tr>
<td>100 bp DNA Ladder</td>
<td>31031</td>
<td>30 ug</td>
<td></td>
</tr>
<tr>
<td>DNA Gel Extraction Kit</td>
<td>31030-50</td>
<td>50 columns</td>
<td>Validated for use with GelRed™ and GelGreen™*</td>
</tr>
<tr>
<td></td>
<td>31030-250</td>
<td>250 columns</td>
<td></td>
</tr>
<tr>
<td>5X TBE Buffer</td>
<td>41006</td>
<td>4 L</td>
<td>Electrophoresis buffer concentrate</td>
</tr>
</tbody>
</table>

* GelRed™ and GelGreen™ also are compatible with popular gel extraction kits and DNA ladders from other suppliers.
**EvaGreen® Dye for qPCR**

EvaGreen® dye is a next-generation DNA-binding dye with features ideal for use in quantitative real-time PCR (qPCR) and other applications. Biotium scientists designed the dye with several essential dye properties in mind, including PCR inhibition, dye safety, stability, and fluorescence spectra of the dye. The results of our efforts is a dye superior to other dyes such as SYBR® Green I for PCR and melt curve analysis.

**Superior PCR performance**

EvaGreen® dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits the use of a relatively high dye concentration without inhibiting PCR. The dye is constructed of two monomeric DNA-binding dyes linked by a flexible spacer. The dimeric dye randomly shifts between an inactive looped conformation that does not bind DNA at low DNA concentrations at the beginning of a PCR reaction, and an active conformation that becomes stabilized as DNA concentration increases during PCR. Consequently, EvaGreen® can be used at relatively high dye concentrations, resulting in improved sensitivity and more accurate melt curve analysis compared to other qPCR dyes such as SYBR® Green. EvaGreen® dye also is compatible with microfluidics-based qPCR platforms and isothermal DNA amplification methods. EvaGreen® is detected using the same instrument settings as SYBR® Green.

An added benefit of EvaGreen® dye is that you can analyze your PCR products by gel electrophoresis without the need to use a DNA gel stain. The EvaGreen® dye in the PCR reaction doubles as a DNA prestain, permitting direct visualization of DNA bands following electrophoresis.

**Safer handling and disposal**

A unique feature of EvaGreen® dye is its safety. Handling and disposal of qPCR master mixes may pose health and environmental risks. For example, SYBR® Green I, a popular PCR dye, has been found to be even more environmentally toxic than ethidium bromide, a well-known mutagen (Ohta et al., 2001). With this in mind, Biotium’s scientists designed EvaGreen® dye such that it cannot cross cell membranes, thus preventing the dye from binding genomic DNA in living cells (Figure 3). Independent labs have confirmed that EvaGreen® dye is nonmutagenic, noncytotoxic and safe to aquatic life, and classified as non-hazardous waste. A detailed safety report for EvaGreen® is available for download from www.biotium.com.

**References**

EvaGreen has been cited in more than 2500 publications to date (Google Scholar search).


EvaGreen is a registered trademark of Biotium, Inc. EvaGreen dye technologies are covered by US patent Nos. 7,601,498, 7,776,567 and other pending US and international patents. AmpliTag Gold and TaqMan are registered trademarks of Roche Molecular Systems, Inc. SYBR is a registered trademark of Invitrogen Corp. Practicing HRM may require a license from Idaho Technologies, Inc.

**FEATURES**

**Safer and more environmentally friendly**

EvaGreen® dye, which has passed California environmental regulation (CCR Title 22) for disposal down the drain.

**High sensitivity**

Unique "release-on-demand" DNA binding mechanism of EvaGreen® dye enables superior PCR and melt curve analysis.

**Rapid hot-start**

Novel chemically-modified hot start Taq, Cheetah™ Taq requires only 2 minutes to activate.

**Compatible with both fast and regular cycling protocols**

**Detected using the same instrument settings as SYBR® Green.**

**Direct visualization of PCR product in gels**

Analyze your PCR product by gel electrophoresis without the need to add another gel stain.

![Figure 1. EvaGreen® dye binds to dsDNA via a "release-on-demand" mechanism.](image)

![Figure 2. EvaGreen® dye for qPCR offers superior sensitivity compared to SYBR® Green.](image)
**Environmental Friendly Dye with Superior qPCR Performance**

**PCR master mixes featuring EvaGreen® dye and Cheetah™ Taq**

Biotium’s qPCR master mixes feature Cheetah™ Taq, our proprietary chemically-modified hot-start DNA polymerase. Unlike AmpliTaq Gold®, which takes 10 minutes or longer to activate, Cheetah™ Taq is fully recovered in 2 minutes with high activity (Figure 4), making it particularly suitable for fast PCR.

**EvaGreen Dye Products for qPCR**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix</td>
<td>Suitable for both qPCR and melt curve analysis, the master mix contains EvaGreen® dye and our superior chemically modified hot start Cheetah™ Taq to deliver unmatched results. Does not contain Rox reference dye.</td>
</tr>
<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix, Low Rox</td>
<td>Fast Plus Master Mix with low concentration of Rox reference dye for use with ABI 7500, 7500 Fast or Stratagene MX4000P, MX3000P, MX3005P instruments.</td>
</tr>
<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix, High Rox</td>
<td>Fast Plus Master Mix with high concentration of Rox reference dye for use with ABI 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, or StepOne plus instruments.</td>
</tr>
<tr>
<td>Fast Probe Master Mix</td>
<td>Contains our fast-activating hot start Cheetah™ Taq and proprietary buffer without DNA binding dye. Suitable for use with TaqMan®-like probes and Fluidigm® qPCR platforms.</td>
</tr>
<tr>
<td>Fast Probe Master Mix, High Rox</td>
<td>Contains our fast-activating hot start Cheetah™ Taq and proprietary buffer without DNA binding dye. Suitable for use with TaqMan®-like probes and Fluidigm® qPCR platforms. With high concentration of Rox reference dye.</td>
</tr>
<tr>
<td>EvaGreen™ Dye, 20X in water</td>
<td>Stand-alone EvaGreen dye for use with your own PCR reaction mix.</td>
</tr>
<tr>
<td>Cheetah™ Taq</td>
<td>Stand-alone chemically-modified hot start Taq polymerase for use with your own PCR reaction mix.</td>
</tr>
<tr>
<td>ROX Reference Dye, 25 uM in TE</td>
<td>Stand-alone ROX reference dye for use with your own PCR reaction mix.</td>
</tr>
</tbody>
</table>

**Ordering Information**

<table>
<thead>
<tr>
<th>Description</th>
<th>Size</th>
<th>Cat. No.</th>
<th>Unit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast EvaGreen® qPCR Master Mix</td>
<td>Trial size, 100 reactions</td>
<td>31003-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td></td>
<td>200 reactions</td>
<td>31003</td>
<td>Each (2 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
<td>31003-1</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>5000 reactions</td>
<td>31003-2</td>
<td>Each (50 x 1 mL)</td>
</tr>
<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix</td>
<td>Trial size, 100 reactions</td>
<td>31020-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td></td>
<td>200 reactions</td>
<td>31020</td>
<td>Each (2 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
<td>31020-1</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>5000 reactions</td>
<td>31020-2</td>
<td>Each (50 x 1 mL)</td>
</tr>
<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix, Low ROX</td>
<td>Trial size, 100 reactions</td>
<td>31014-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td></td>
<td>200 reactions</td>
<td>31014</td>
<td>Each (2 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
<td>31014-1</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>5000 reactions</td>
<td>31014-2</td>
<td>Each (50 x 1 mL)</td>
</tr>
<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix, High ROX</td>
<td>Trial size, 100 reactions</td>
<td>31015-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td></td>
<td>200 reactions</td>
<td>31015</td>
<td>Each (2 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
<td>31015-1</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>5000 reactions</td>
<td>31015-2</td>
<td>Each (50 x 1 mL)</td>
</tr>
<tr>
<td>Fast Probe Master Mix</td>
<td>Trial size, 100 reactions</td>
<td>31005-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td></td>
<td>200 reactions</td>
<td>31005</td>
<td>Each (2 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
<td>31005-1</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>5000 reactions</td>
<td>31005-2</td>
<td>Each (50 x 1 mL)</td>
</tr>
<tr>
<td>Fast Probe Master Mix, High ROX</td>
<td>Trial size, 100 reactions</td>
<td>31016-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td></td>
<td>200 reactions</td>
<td>31016</td>
<td>Each (2 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
<td>31016-1</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td></td>
<td>5000 reactions</td>
<td>31016-2</td>
<td>Each (50 x 1 mL)</td>
</tr>
<tr>
<td>EvaGreen® Dye, 20X in Water, Trial Size</td>
<td>1 mL</td>
<td>31000-T</td>
<td>Each (1 mL)</td>
</tr>
<tr>
<td>EvaGreen® Dye, 20X in Water</td>
<td>5 x 1 mL</td>
<td>31000</td>
<td>Each (5 x 1 mL)</td>
</tr>
<tr>
<td>Cheetah™ Taq</td>
<td>500 U</td>
<td>29050</td>
<td>Each (500 U)</td>
</tr>
<tr>
<td>ROX Passive Reference Dye, 25 uM in TE</td>
<td>5 x 1 mL</td>
<td>29052</td>
<td>Each (5 x 1 mL)</td>
</tr>
</tbody>
</table>

**Figure 3.** EvaGreen® dye is safer because it cannot penetrate cell membranes to bind DNA in living cells. Comparison of cell membrane permeability between EvaGreen® dye and SYBR® Green I. HeLa cells were incubated with SYBR® Green I or EvaGreen® dye (1.2 uM final concentration for both dyes) at 37°C. SYBR® Green I rapidly entered cells and stained nuclei, while GelGreen™ was unable to penetrate cell membranes, demonstrated by the absence of nuclear staining. For more information, download the EvaGreen Safety Report at www.biotium.com.

**Figure 4.** Cheetah™ Taq requires only 2 minutes of hot-start for full recovery of activity. Comparison of hot-start recovery of polymerase activity for Cheetah™ Taq and AmpliTaq Gold® following incubation at 95°C in 50 mM pH 8.0 Tris.
DNA Polymerase Modification and Activity Kits

HotStart Polymerase Modification Kit
- Hot-start any thermostable DNA polymerase with the same patented technology used in Cheetah Taq™
- Prevent primer-dimer formation and mis-priming
- Fast hot-start activation, 2 minutes at 95°C (see p. 5)
- Includes Lumitein™ protein gel stain (p. 10) to confirm modification by PAGE gel electrophoresis (Fig. 1).

New! WarmStart™ Enzyme Modification Kit
- Novel chemical modifier for non-thermostable enzymes
- Turn off enzyme activity at room temperature
- Regain activity by heating to 45-60°C

Applications:
- Reverse transcriptase
- Bst DNA polymerase
- E. coli DNA polymerase I
- Restriction enzymes
- Nucleases
- Proteases

EvaEZ™ Fluorometric Polymerase Activity Assay
- Non-radioactive assay for DNA acid polymerases
- Uses safe and sensitive EvaGreen™ dye
- Assay enzyme activity between 4°C-75°C.
- Confirm hot-start or warm-start modification of DNA polymerases (Fig. 1)

Determine polymerase activity of:
- Taq, Pfu, Vent®, Phusion®
- AMV
- Bst
- Phi29
- MMLV, SuperScript®
- T4 & T7 DNA polymerases
- E. coli DNA polymerase I, Klenow fragment

Table 1. Ordering Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
<th>Unit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>HotStart Polymerase Modification Kit</td>
<td>29054-T</td>
<td>Trial size, sufficient to modify 0.1 mg polymerase</td>
</tr>
<tr>
<td></td>
<td>29054</td>
<td>Sufficient to modify 0.5 mg polymerase</td>
</tr>
<tr>
<td>WarmStart™ Enzyme Modification Kit</td>
<td>29053-T</td>
<td>Trial size, sufficient to modify 0.1 mg protein</td>
</tr>
<tr>
<td></td>
<td>29053</td>
<td>Sufficient to modify 0.5 mg protein</td>
</tr>
<tr>
<td>EvaEZ™ Fluorometric Polymerase Activity Assay Kit</td>
<td>29051</td>
<td>2 x 1 mL (200 reactions)</td>
</tr>
</tbody>
</table>

Figure 1. A. EvaEZ Polymerase Activity assay showing Taq polymerase activity without modification (a), after modification with the HotStart Polymerase Modification Kit (b), and after reactivation of the modified enzyme by heating to 90°C (c). B. Lumitein-stained non-denaturing acrylamide gel showing increased electrophoretic mobility of Taq polymerase after modification (lane 2) compared to unmodified enzyme (lane 1).
the ability to selectively and sensitively detect viable bacteria in the presence of dead bacteria is of vital importance for many practical applications, including safety inspection of food products, drinking water quality control, and medical diagnosis. The traditional detection method based on bacterial culturing is time-consuming and insensitive. Detection based on PCR is a rapid and highly sensitive alternative method. However, PCR methods cannot distinguish live from dead cells, making interpretation of any analytical results difficult. The novel DNA-modifying dye propidium monoazide (PMA™) developed by Biotium overcomes this problem. Pre-treating a sample with PMA™ prior to PCR analysis permits one to selectively detect only viable bacteria in a highly sensitive and reliable manner.

PMA™ is a photo-reactive dye with a high affinity for DNA. The dye intercalates into dsDNA and forms a covalent linkage upon exposure to intense visible light, resulting in chemically modified DNA, which cannot be amplified by PCR (Figure 1). Because PMA™ is designed to be cell membrane-impermeable, when a sample comprising both live and dead bacteria is treated with PMA™, only dead bacteria are susceptible to DNA modification due to compromised cell membranes. Thus, subsequent DNA extraction followed by qPCR permits selective detection of live cells. The number of dead bacteria in a sample is proportional to the delay in PCR threshold cycle of a PMA-treated sample compared to the threshold cycle of an untreated sample. Conventional PCR and gel analysis can be performed with PMA, as well, using the amount of DNA amplified by the PCR reaction as an indicator of the number of viable cells in the sample.

PMA™-qPCR method also have been used with non-bacterial organisms such as yeast, fungi, amoebae, and viruses.

Selected References:


AccuBlue™ and AccuClear™

dsDNA Quantitation Assays

AccuBlue™ and AccuClear™ dsDNA quantitation assays allow precise quantitation of purified dsDNA samples across a wide range of concentrations. Unlike absorbance-based nucleic acid quantitation, fluorescent DNA binding dyes are highly sensitive and selective for double-stranded DNA and provide a more accurate DNA concentration in the presence of contaminating RNA and other common contaminants including free nucleotides, protein, detergents and salts. Biotium offers three dsDNA quantitation kits for different instruments and sample concentration ranges. The kits also differ in their tolerance of various contaminants; download the product protocols at www.biotium.com for details. The AccuClear™ Ultra High Sensitivity assay is based on a novel, next-generation DNA binding dye which offers unrivaled sensitivity and dynamic range compared to other DNA dyes. The kits are compatible with Biotium’s compact and affordable AccuLite™ Mini Fluorometers.

---

**AccuBlue™ Broad Range**
- Linear range: 2-2000 ng dsDNA
- Blue fluorescence (Ex/Em: 350/460 nm)
- Assay can be extended to 4000 ng dsDNA with minor loss of linearity
- Blue fluorescence detection, compatible with fluorescence microplate reader and Biotium’s AccuLite™ 350 handheld fluorometer

**AccuBlue™ High Sensitivity**
- Linear range: 0.2-100 ng dsDNA
- Green fluorescence (Ex/Em: 485/530 nm)
- Membrane-impermeable dye is non-toxic and non-mutagenic, for safer handling and easy disposal
- Green fluorescence detection compatible with fluorescence microplate reader, NanoDrop® fluorospectrophotometer, Biotium’s AccuLite™ 470 handheld fluorometer and other handheld fluorometers like Qubit® and QuantiFluor-P™

**AccuClear™ Ultra High Sensitivity**
- Linear range: 0.03-250 ng dsDNA
- Green fluorescence (Ex/Em: 460/507 nm)
- Unrivaled sensitivity and dynamic range
- Novel green fluorescent dye is a perfect match for blue LED excitation sources
- Green fluorescence detection compatible with fluorescence microplate reader, Biotium’s AccuLite™ 470 handheld fluorometer, or NanoDrop® fluorospectrophotometer

---

**AccuLite™ Mini Fluorometers**
- Accept 200 ul PCR tubes
- LCD touch-screen display
- USB interface for data management
- Compact (185 x 90 x 35 mm, 10 oz)
- Greater than 6 logs of dynamic range
- Include programs for use with AccuBlue and AccuClear dsDNA quantitation assays
- Power Supply: 4 AA batteries or a 5V DC adapter
- AccuLite 350: 365-370 nm LED excitation 460+/-20 nm emission
- AccuLite 470: 465-475 nm LED excitation 540+/-30 nm emission

---

Figure 1. AccuLite™ Mini Fluorometers
Superior sensitivity and broad dynamic range

Figure 2: Triplicate samples of calf thymus dsDNA, mouse liver RNA or salmon sperm ssDNA were assayed with the AccuBlue High Sensitivity dsDNA Quantitation Kit. The assay is selective for double-stranded DNA over RNA or single-stranded DNA.

Figure 3: Standard curve of calf thymus DNA assayed using AccuBlue Broad Range Kit and read on a microplate reader (Ex/Em 350/460). Inset shows the lower end of the titration.

Figure 4: Two-fold dilutions of calf thymus DNA were assayed using AccuBlue or Quant-iT Broad Range assay kits. AccuBlue has a wider dynamic range than the Quant-iT.

Figure 5: Standard curve of calf thymus DNA assayed using the AccuBlue High Sensitivity Kit and read on a microplate reader (Ex/Em 485/530). Inset shows the lower end of the titration.

Figure 6: Standard curve of calf thymus DNA assayed using the AccuClear Ultra High Sensitivity Kit and read on a microplate reader (Ex/Em 460/507). Inset shows the lower end of the titration.

Figure 7: The AccuBlue High Sensitivity (HS) reagent and PicoGreen were diluted to working concentrations in HeLa cell cultures and incubated for 30 minutes. PicoGreen and the Quant-iT High Sensitivity reagent (Invitrogen) readily bind nuclear DNA while no nuclear staining is evident with the AccuBlue HS reagent, illustrating its overall safety and lower toxicity due to its membrane impermeability.

**Ordering Information**

<table>
<thead>
<tr>
<th>Linear Range</th>
<th>Description</th>
<th>Cat. No.</th>
<th>Unit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad Range</td>
<td>AccuBlue Broad Range dsDNA Quantitation Solution, Trial Size</td>
<td>31009-T</td>
<td>Each, 200 Assays</td>
</tr>
<tr>
<td>2-2000 ng</td>
<td>AccuBlue Broad Range dsDNA Quantitation Solution</td>
<td>31009</td>
<td>Each, 1000 Assays</td>
</tr>
<tr>
<td></td>
<td>AccuBlue Broad Range dsDNA Quantitation Kit with DNA Standard, Trial Size</td>
<td>31007-T</td>
<td>Each, 200 Assays</td>
</tr>
<tr>
<td></td>
<td>AccuBlue Broad Range dsDNA Quantitation Kit with 9 DNA Standards</td>
<td>31007</td>
<td>Each, 1000 Assays</td>
</tr>
<tr>
<td>High Sensitivity</td>
<td>AccuBlue High Sensitivity dsDNA Quantitation Solution, Trial Size</td>
<td>31008-T</td>
<td>Each, 200 Assays</td>
</tr>
<tr>
<td>0.2-100 ng</td>
<td>AccuBlue High Sensitivity dsDNA Quantitation Solution</td>
<td>31008</td>
<td>Each, 1000 Assays</td>
</tr>
<tr>
<td></td>
<td>AccuBlue High Sensitivity dsDNA Quantitation Kit with DNA Standard, Trial Size</td>
<td>31006-T</td>
<td>Each, 200 Assays</td>
</tr>
<tr>
<td></td>
<td>AccuBlue High Sensitivity dsDNA Quantitation Kit with 8 DNA Standards</td>
<td>31006</td>
<td>Each, 1000 Assays</td>
</tr>
<tr>
<td>Ultra High Sensitivity</td>
<td>AccuClear Ultra High Sensitivity dsDNA Quantitation Solution, Trial Size</td>
<td>31027-T</td>
<td>Each, 200 Assays</td>
</tr>
<tr>
<td>0.03-250 ng</td>
<td>AccuClear Ultra High Sensitivity dsDNA Quantitation Solution</td>
<td>31027</td>
<td>Each, 1000 Assays</td>
</tr>
<tr>
<td></td>
<td>AccuClear Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards</td>
<td>31028</td>
<td>Each, 1000 Assays</td>
</tr>
<tr>
<td></td>
<td>AccuClear Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard</td>
<td>31029</td>
<td>Each, 4000 Assays</td>
</tr>
<tr>
<td>Stand-alone dsDNA standard sets</td>
<td>AccuBlue Broad Range dsDNA Standards, Set of Nine (0-200 ng/L calf thymus dsDNA)</td>
<td>31007C</td>
<td>0.5 mL each</td>
</tr>
<tr>
<td></td>
<td>AccuBlue High Sensitivity dsDNA Standards, Set of Eight (0-10 ng/L calf thymus dsDNA)</td>
<td>31006C</td>
<td>0.5 mL each</td>
</tr>
<tr>
<td>AccuLite Mini Fluorometers</td>
<td>AccuLite 350 Mini Fluorometer</td>
<td>E90000</td>
<td>Each</td>
</tr>
<tr>
<td></td>
<td>AccuLite 470 Mini Fluorometer</td>
<td>E90001</td>
<td>Each</td>
</tr>
</tbody>
</table>
**Lumitein™ Protein Gel Stain**

**Ultra Sensitive, Rapid PAGE Gel Staining**

---

**FEATURES**

**Highly sensitive**
At least as sensitive as silver stain, allowing detection of as little as 1 ng protein or less (Figure 1A).

**Simple & fast staining**
Fixation and staining is combined in a single step, for results in 30-90 minutes. Gels can be left in staining solution longer than 90 minutes without overstaining. Native gels can be stained after a brief soak in SDS/acetic acid.

**Compatible with common instruments**
Image using a UV light box, Dark Reader®, or laser scanner.

**Wide linear detection range**
At least three orders of magnitude.

**Compatible with downstream analysis**
Compatible with mass spectroscopy and Edman peptide sequencing (Figure 1B).

**Economical and convenient**
Available as a 100X concentrated solution to reduce manufacturing cost and shipping cost, or as a ready-to-use 1X staining solution for convenience.

**Highly stable**
100X concentrate and 1X working solution are stable at room temperature for at least 1 year.

---

**Selected References**


---

**Table 1. Comparison of standard staining protocols**

<table>
<thead>
<tr>
<th>Protocol Step</th>
<th>SYPRO® Ruby</th>
<th>Lumitein™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation step 1</td>
<td>15 min. 50% methanol/7.5% acetic acid</td>
<td>None</td>
</tr>
<tr>
<td>Fixation step 2</td>
<td>15 min. 50% methanol/7.5% acetic acid</td>
<td>None</td>
</tr>
<tr>
<td>Staining</td>
<td>Overnight</td>
<td>30-90 min.</td>
</tr>
<tr>
<td>Destaining</td>
<td>30 min. 10% methanol/7% acetic acid</td>
<td>No destaining required</td>
</tr>
<tr>
<td>Optional: 5 min. in 30% methanol/15% acetic acid or 20 min. in water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinse 1</td>
<td>5 min. water</td>
<td>Single 5 min. water rinse</td>
</tr>
<tr>
<td>Rinse 2</td>
<td>5 min. water</td>
<td></td>
</tr>
<tr>
<td>Total time</td>
<td>More than 16 hours</td>
<td>2 hours or less</td>
</tr>
</tbody>
</table>

**Ordering Information**

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumitein™ Protein Gel Stain, 1X</td>
<td>21001</td>
<td>200 mL</td>
</tr>
<tr>
<td>21001-1</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>21001-2</td>
<td>5 x 1 L</td>
<td></td>
</tr>
<tr>
<td>Lumitein™ Protein Gel Stain, 100X</td>
<td>21002</td>
<td>2 mL</td>
</tr>
<tr>
<td>21002-1</td>
<td>10 mL</td>
<td></td>
</tr>
<tr>
<td>21002-2</td>
<td>50 mL</td>
<td></td>
</tr>
</tbody>
</table>

Lumitein and its related technologies are covered by pending US and international patents. Lumitein is a trademark of Bio-Tech, Inc. Dark Reader is a registered trademark of Clare Chemical Research; ImageQuant and Typhoon Trio are trademarks of GE Healthcare; Precision Plus Protein is a trademark of Bio-Rad Laboratories; SYPRO is a registered trademark of Molecular Probes, Inc.
AccuOrange™ Protein Quantitation Kit

Highly sensitive, fluorometric protein assay

AccuOrange™ Protein Quantitation Kit is a highly sensitive fluorescence-based assay for quantitating purified protein samples in 96-well format. The detection range of the assay is 0.1-15 ug/mL protein. AccuOrange is much more sensitive than traditional protein quantitation assays such as BCA, Bradford and Lowry, and shows superior linearity and reproducibility compared to the NanoOrange® protein quantitation assay (Figure 2). The assay shows minimal variability between different proteins, and has stable fluorescence signal for up to 16 hours.

AccuOrange is recommended for quantitating purified protein or antibody samples. The tolerance of the AccuOrange assay to salts, buffers, detergents, and other chemicals is shown in Table 1. The AccuOrange assay has low tolerance for non-ionic detergents, and is not recommended for use with cell lysates containing Triton X-100, sodium deoxycholate, CHAPs, or other non-ionic detergents. The assay can tolerate up to 0.01% SDS (final concentration in assay).

Table 1. Comparison of AccuOrange™ with other protein quantitation assays

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Detection Range (microplate assay)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuOrange™</td>
<td>0.1-15 ug/mL</td>
<td>• Fluorescence detection (480/598 nm) • Highly linear • Signal stable for at least 16 hours • Compatible with reducing agents • Not compatible with detergents</td>
</tr>
<tr>
<td>NanoOrange®</td>
<td>0.1-10 ug/mL</td>
<td>• Fluorescence detection (470/570 nm) • Non-linear • Fluorescence stable for 6 hours • Compatible with reducing agents • Not compatible with detergents</td>
</tr>
<tr>
<td>Modified Lowry</td>
<td>1-1500 ug/mL</td>
<td>• Absorbance detection (750 nm) • Non-linear • Not compatible with reducing agents • Not compatible with detergents</td>
</tr>
<tr>
<td>BCA</td>
<td>20-2000 ug/mL</td>
<td>• Absorbance detection (562 nm) • Highly linear • Signal not stable over time • Not compatible with reducing agents • Compatible with detergents</td>
</tr>
<tr>
<td>Bradford (Coomassie)</td>
<td>50-500 ug/mL</td>
<td>• Absorbance detection (595 nm) • Signal not stable over time • Non-linear • Compatible with reducing agents • Not compatible with detergents</td>
</tr>
<tr>
<td>Pierce® 660 nm</td>
<td>50-2000 ug/mL</td>
<td>• Absorbance detection (660 nm) • Non-linear • Compatible with reducing agents • Compatible with detergents</td>
</tr>
<tr>
<td>A&lt;sub&gt;280&lt;/sub&gt;</td>
<td>50-2000 ug/mL</td>
<td>• Absorbance detection (280 nm) • High protein-protein variability • Contaminants such as nucleic acids can affect results</td>
</tr>
</tbody>
</table>

NanoOrange is a registered trademark of Molecular Probes Inc.

Figure 1. AccuOrange™ shows better linearity and reproducibility compared to NanoOrange® Protein Quantitation assay. BSA titration was performed in triplicate using AccuOrange Protein Quantitation Kit or NanoOrange Protein Quantitation Kit from Life Technologies according to manufacturer’s protocol and read on a microplate reader at the recommended wavelengths for each assay. Inset shows the lower end of the curve. Error bars represent standard deviation of the mean for triplicate samples.

Ordering Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuOrange™ Protein Quantitation Kit</td>
<td>30071-T</td>
<td>200 assays</td>
</tr>
<tr>
<td></td>
<td>30071</td>
<td>2000 assays</td>
</tr>
</tbody>
</table>

NanoOrange is a registered trademark of Molecular Probes Inc.