Molecular Biology

PCR Reagents
Cheetah™ hot-start Taq and EvaGreen® PCR dye ... p. 2
Forget-Me-Not™ qPCR Master Mixes ... p. 3
HotStart and WarmStart™ polymerase modification kits ... p. 4
EvaEZ™ DNA Polymerase Activity Assay ... p. 4

DNA and Protein Quantitation
AccuOrange™ protein quantitation assay ... p. 5
AccuClear®, AccuBlue® and AccuGreen™ DNA quantitation assays ... pp. 6-7

DNA and Protein Gel Stains
GelRed® and GelGreen® nucleic acid gel stains ... pp. 8-9
DNAzure® visible nucleic acid stain ... p. 10
Gel-Bright™ LED Gel Illuminator ... p. 10
One-Step protein gel stains ... p. 11

Microbe Detection by PCR
PMAxx™ and PMA for viability PCR ... p. 12-13
PMA-Lite™ and Glo-Plate™ LED photolysis devices ... p. 13

DNA purification kits
RNAstorm™ and DNAstorm™ FFPE extraction kits ... p. 14

Other Biotium products ... p. 15

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Biotium

Cheetah™ Taq & EvaGreen® qPCR Dye

EvaGreen® Features

- High sensitivity, bright DNA-binding dye
- Detected in the SYBR® Green channel
- Less PCR inhibition than SYBR® Green
- Direct visualization of PCR product in gels
- Safer and more environmentally friendly
- The only qPCR dye used in droplet digital PCR (ddPCR)
- The best dye for high resolution melt curve analysis (HRM)

Fast EvaGreen® qPCR Master Mixes
Convenient, ready-to-use qPCR Master Mixes feature bright and safe EvaGreen® dye and our proprietary Cheetah™ Taq hot-start polymerase.

We also offer Fast Probe Master Mixes featuring Cheetah™ Taq without dye, for probe-based PCR reactions. Our Fast Probe Master Mixes are used extensively in microfluidics-based PCR platforms such as Fluidigms instruments.

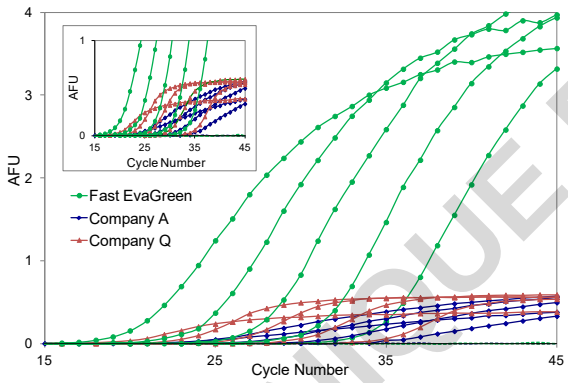


Figure 1. EvaGreen® PCR dye is bright and sensitive. Comparison between an EvaGreen® qPCR Master Mix from Biotium and two fast SYBR® Green master mixes from two leading companies (company A and company Q) under similar condition. The inset is an enlarged view of the area near the baseline for better viewing the curve patterns of the weaker signals of the two SYBR®-based master mixes. Amplicon: ATPG fragment of human genomic DNA; instrument: ABI 7900 Fast.

Cheetah™ Taq Hot-Start DNA Polymerase
Cheetah™ Taq is Biotium's 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, making it particularly suitable for fast PCR.

Cheetah™ Taq Features

- Proprietary chemically-modified hot-start Taq polymerase
- Hot-start-modified enzyme is completely inhibited on the bench, but fully active after just 2 minutes of heating
- Ideal for fast PCR reactions
- Used in all of Biotiums qPCR Master Mixes

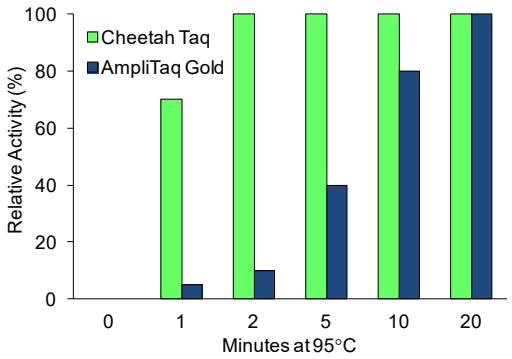


Figure 2. Cheetah™ Taq requires only 2 minutes of hot-start for full recovery of activity. Comparison of hot-start recovery of polymerase activity for Cheetah™ Taq and AmpliTaq Gold® following incubation at 95°C in 50 mM pH 8.0 Tris.

Fast EvaGreen® and Fast Probe Master Mixes

Cat. #	Description	Size
31003-T	Fast EvaGreen® qPCR Master Mix	Trial size, 1 mL
31003		2 x 1 mL
31003-1		5 x 1 mL
31005-T	Fast Probe Master Mix	Trial size, 1 mL
31005		2 x 1 mL
31005-1		5 x 1 mL
31016-T	Fast Probe Master Mix, High ROX	Trial size, 1 mL
31016		2 x 1 mL
31016-1		5 x 1 mL

PCR reagents sold separately

Cat. #	Description	Size
31000-T	EvaGreen® Dye, 20X in Water	Trial size, 1 mL
31000		5 x 1 mL
31019	EvaGreen® Dye, 2000X in DMSO	50 uL
29050	Cheetah™ Taq	500 U
40054	dNTP mix, 10 mM each	5 x 1 mL
40052	dNTP set, 100 mM each	Set of 1 mL each
29052	ROX Passive Reference Dye, 25 uM in TE	5 x 1 mL

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EvaGreen is a registered trademark of Biotium, Inc. EvaGreen dye and Cheetah Taq technologies are covered by granted US and/or international patents. Amplitaq Gold is a registered trademarks of Roche Molecular Systems, Inc. SYBR is a registered trademark of Thermo Fisher Scientific. Practicing HRM may require a license from Idaho Technologies, Inc.

Forget-Me-Not™ EvaGreen® qPCR Master Mixes

Forget-Me-Not™ EvaGreen® qPCR Master Mixes are hot-start EvaGreen® dye-based master mixes for use in real time PCR applications and DNA melt curve analysis. They are available in no, low or high ROX formulations.

Master Mixes with 2-Color Tracking

The Forget-Me-Not™ EvaGreen® qPCR Master Mixes with 2-Color Tracking feature a unique combination of a master mix containing a low concentration of blue dye, plus a DNA template buffer containing a higher concentration of blue dye. When you add the 2X Forget-Me-Not™ Master Mix to your reaction tube, it appears light blue. Then, when you add template containing Forget-Me-Not™ Template Buffer to the reaction, the color turns dark blue. Forget-Me-Not™ allows you to see at a glance whether you forgot to add master mix or template to any of your reactions, so you can catch pipetting mistakes and avoid wasting time, reagents, and your precious DNA samples. The 2X Forget-Me-Not™ Master Mix also can be used without the Template Buffer if you prefer.

Two Color Tracking to Minimize Errors



Figure 1. PCR tubes containing Forget-Me-Not™ qPCR Master Mix (1X) on top and Forget-Me-Not qPCR Master Mix (1X) plus DNA template in Template Buffer on the bottom.

Superior Melt Curve Analysis

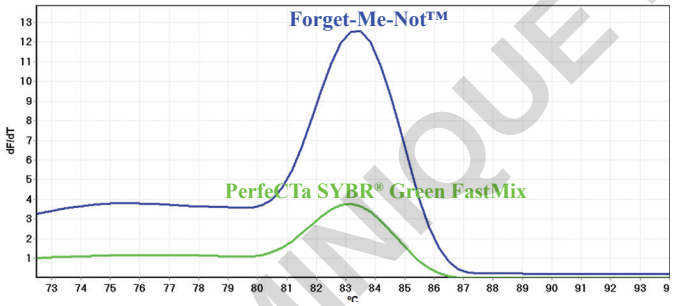


Figure 2. Melt curve analysis comparing Forget-Me-Not™ (blue line) with Quanta's PerfeCTa SYBR® Green FastMix (green line).

Brighter signal than SYBR® Green

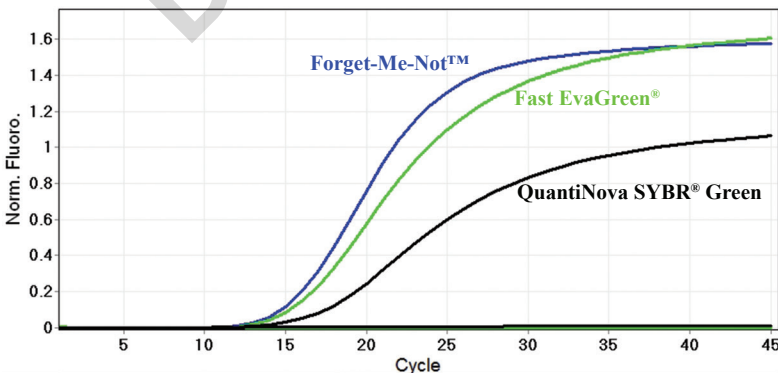


Figure 3. Real-time PCR data comparing Forget-Me-Not™ (blue line) with Biotium's Fast EvaGreen® (green line) and Qiagen's QuantiNova® SYBR® Green (black line) master mixes. Amplification curves on linear scale. EvaGreen® dye-based master mixes yield higher signal compared to the SYBR® Green-based mix.

Forget-Me-Not™ Universal Probe Master Mix

Forget-Me-Not™ Universal Probe Master Mix is a high-performance product for fluorescent probe-based PCR applications, including quantitation and SNP genotyping. This kit is suitable for all fluorescent probe-based technologies, including hydrolysis probes (such as TaqMan® and dual-labeled BHQ® probes) and displacement probes (like Molecular Beacons). Forget-Me-Not™ Universal Probe Master Mix shows excellent concordance of results in singleplex and multiplex reactions, has broad instrument compatibility, and can be used in both standard and fast protocols.

Forget-Me-Not™ Universal Probe Master Mix comes with an optional blue tracking buffer containing an inert blue dye. You have the choice of adding Tracking Buffer to the master mix, to the DNA template, or not to use the tracking buffer in your reactions.

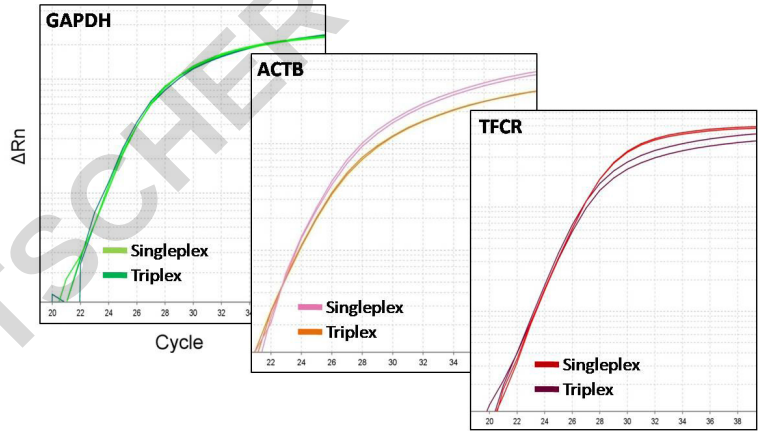


Figure 4. Singleplex and triplex PCR of GAPDH, ACTB and TFCR from human genomic DNA. Reactions contained 20 ng of human genomic DNA, 400 nM of each primer, and 200 nM of each hydrolysis probe (GAPDH-FAM/BHQ®-1, ACTB-CAL Fluor® Orange 560/BHQ®-1, TFCR-CAL Fluor Red 610/ BHQ®-2).

Forget-Me-Not™ Master Mixes

Cat. #	Description	Size
31041-T	Forget-Me-Not™ EvaGreen® qPCR Master Mix with 2-Color Tracking	Trial size, 1 mL
31041-1		5 x 1 mL
31041-20mL		2 x 10 mL
31042-T	Forget-Me-Not™ EvaGreen® qPCR Master Mix with 2-Color Tracking, with separate ROX	Trial size, 1 mL
31042-1		5 x 1 mL
31042-20mL		2 x 10 mL
31045-T	Forget-Me-Not™ EvaGreen® qPCR Master Mix (low ROX)	Trial size, 1 mL
31045-5mL		5 x 1 mL
31045-20mL		2 x 10 mL
31046-T	Forget-Me-Not™ EvaGreen® qPCR Master Mix (high ROX)	Trial size, 1 mL
31046-5mL		5 x 1 mL
31046-20mL		2 x 10 mL
31043-T	Forget-Me-Not™ Universal Probe Master Mix	Trial size, 1 mL
31043-1		5 x 1 mL
31044-T	Forget-Me-Not™ Universal Probe Master Mix with ROX	Trial size, 1 mL
31044-1		5 x 1 mL

TaqMan is a registered trademark of Thermo Fisher Scientific. Quantinova is a registed trademark of Qiagen. BHQ and Cal Fluor are registered trademarks of Biosearch Technologies.

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DNA Polymerase Modification and Activity Kits

HotStart Polymerase Modification Kit
HotStart Polymerase Modification Kit provides an easy way to reversibly modify lysine residues of thermostable DNA polymerases, rendering the enzyme inactive. The modification is reversed after heating to >90°C. HotStart modification of DNA polymerase for PCR prevents amplification of non-specific PCR products due to low stringency annealing of primers at low temperature during reaction assembly.

WarmStart™ Enzyme Modification Kit
The WarmStart™ Enzyme Modification Kit allows temperature control of enzyme activity to be applied to non-thermostable enzymes like reverse transcriptase. Like HotStart, the enzyme is inactivated by modification of lysine residues with a chemical modifier. Protein activity is restored by heating to 45°C or higher.

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
- Includes Lumitein™ protein gel stain to confirm modification by PAGE gel electrophoresis

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

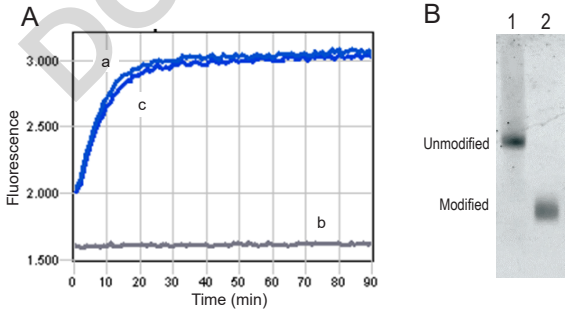


Figure 1. A. EvaEZ™ Polymerase Activity assay showing Taq polymerase activity without modification (a), after modification with the HotStart Polymerase Modification Kit (b), and after reactivation of the modified enzyme by heating to 90°C (c). B. Lumitein-stained non-denaturing acrylamide gel showing increased electrophoretic mobility of Taq polymerase after modification (lane 2) compared to unmodified enzyme (lane 1).

EvaEZ™ Fluorometric Polymerase Activity Assay
EvaEZ™ Fluorometric Polymerase Activity Assay Kit provides an easy and accurate way to determine the activity of a nucleic acid polymerase without using radioisotopes. It contains EvaGreen® dye together with a primed template, dNTPs, and $MgCl_2$ in a Tris buffer. In the presence of DNA polymerase activity, the primer will be extended to form a double stranded product that can bind EvaGreen® dye, resulting in an increase of fluorescence. The rate of increase of fluorescence is positively correlated to the activity of polymerase.

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

Determine polymerase activity of:

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

Enzyme Modification Kits and Activity Assays

Cat. #	Description	Size
29054-T	HotStart Polymerase Modification Kit	Trial size, for 0.1 mg polymerase
29053-T	WarmStart™ Enzyme Modification Kit	Trial size, for 0.1 mg polymerase
29051	EvaEZ™ Fluorometric Polymerase Activity Assay	2 x 1 mL

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Phusion and SuperScript are registered trademarks of Thermo Fisher Scientific. Vent is a registered trademark of New England BioLabs. HotStart and WarmStart modification kits are covered by granted and/or pending U.S. and/or international patents.

AccuOrange™ Protein Quantitation Assay

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 1). 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 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).

AccuOrange™ Features

- Ex/Em: 480/598 nm
- Linear detection range: 0.1-15 ug/mL protein
- 200 uL microplate assay
- Minimal protein-protein variation
- Fluorescence signal stable for at least 16 hours
- Compatible with reducing agents, amino acids, nucleic acids, and imidazole
- For use with purified protein or antibody samples

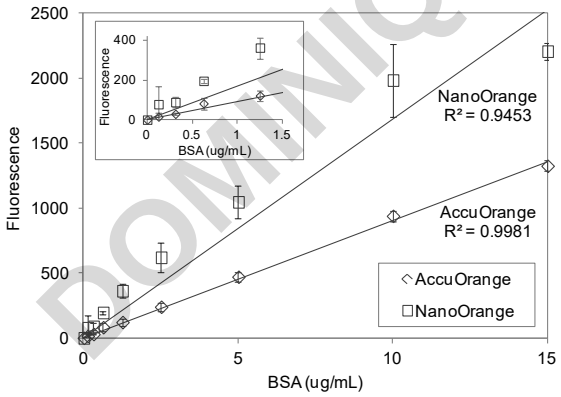


Figure 1. AccuOrange™ shows better linearity and reproducibility compared to NanoOrange® Protein Quantitation assay. BSA titration was performed in triplicate using AccuOrange™ Protein Quantitation Kit or NanoOrange® Protein Quantitation Kit from Life Technologies according to manufacturer's protocol 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.

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

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

Ordering Information

Description	Cat. #	Size
AccuOrange™ Protein Quantitation Kit	30071-T	200 assays
	30071	2000 assays

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NanoOrange is a registered trademark of ThermoFisher

DNA Quantitation Assays

The most sensitive and accurate dsDNA quantitation reagents on the market!

AccuBlue®, AccuClear® and AccuGreen™ dsDNA quantitation assays allow precise quantitation of purified dsDNA samples across a wide range of concentrations and a variety of fluorescence detection instruments. 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 dsDNA quantitiation kits and solutions for different instruments and sample concentration ranges. Some of our kits offer unrivaled sensitivity or linear ranges. To choose the DNA quantitation assay that is right for you, see the linear detection ranges and features table below.

Microplate reader assay features

- Quantify many samples and replicates at once, for better accuracy
- Microplate readers have the highest sensitivity and adjustable excitation and emission settings
- Accurately detect as little as 1 pg DNA with AccuBlue® NextGen, the most sensitive assay on the market
- AccuClear® assay has a very broad dynamic range, allowing quantitation of nearly any sample
- AccuBlue® High Sensitivity is non-mutagenic, safer than PicoGreen®*

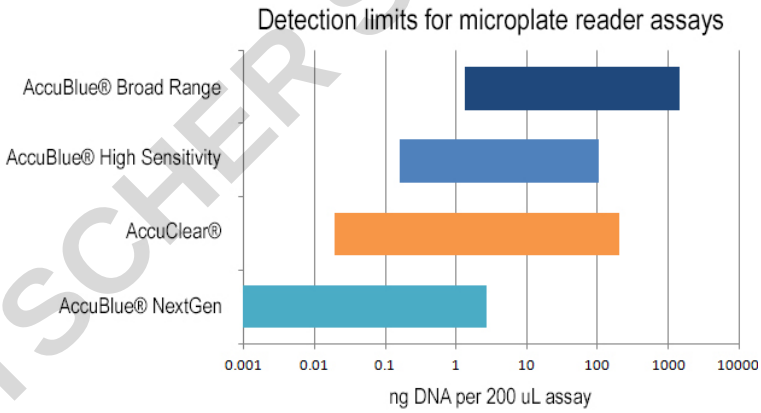


Figure 1. Chart comparing the linear detection ranges for each dsDNA Quantitation Kit for use with fluorescent plate readers. The units are ng of dsDNA per 200 uL reaction. Shown in log scale.

AccuBlue® NextGen

- Linear range: 2.5 pg-3 ng dsDNA
- Green fluorescence (Ex/Em: 468/507 nm)
- The most sensitive and accurate dsDNA quantitation reagent on the market
- Ideal for quantification of low-concentration or precious samples
- For detection on fluorescence microplate readers

AccuClear® Ultra High Sensitivity

- Linear range: 0.03-250 ng dsDNA
- Green fluorescence (Ex/Em: 468/507 nm)
- First-class sensitivity and dynamic range
- Novel green fluorescent dye is a perfect match for blue LED excitation sources
- For detection on fluorescence microplate readers

AccuBlue® High Sensitivity

- Linear range: 0.2-100 ng dsDNA
- Green fluorescence (Ex/Em: 500/530 nm)
- Membrane-impermeable dye is non-toxic and non-mutagenic, for safer handling and easy disposal*
- For detection on fluorescence microplate readers

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
- For detection on fluorescence microplate readers

*AccuBlue® High Sensitivity has been demonstrated by independent labs to be safer than PicoGreen®. Lower toxicity is due to reduced membrane permeability.

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AccuGreen™ for Qubit® features

- Designed for use with Qubit® reader dsDNA programs
- Direct replacements for the Qubit® assay kits
- Large cost savings compared to Qubit® kits

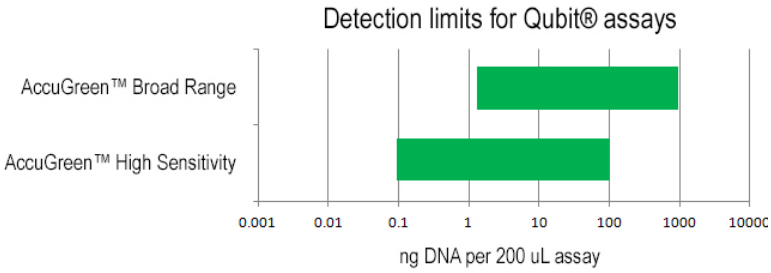


Figure 2. Chart comparing the linear detection ranges for each dsDNA Quantitation Kit for use with the Qubit® reader. The units are ng of dsDNA per 200 uL reaction. Shown in log scale.

AccuGreen™ High Sensitivity (for Qubit®)

- Linear range: 0.1-100 ng dsDNA
- Green fluorescence (Ex/Em: 509/530 nm)
- Designed for detection on the Qubit® fluorometer
- Direct replacement for the Qubit® dsDNA HS Assay Kit

AccuGreen™ Broad Range (for Qubit®)

- Linear range: 2-1000 ng dsDNA
- Green fluorescence (Ex/Em: 500/530 nm)
- Designed for detection on the Qubit® fluorometer
- Direct replacement for the Qubit® dsDNA BR Assay Kit

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Ordering Information

Linear Range	Description	Cat. #	Size
Most Sensitive	AccuBlue® NextGen dsDNA Quantitation Kit with 1 DNA Standard	31060, 31060-T	1000, 200 assays
2.5-3000 pg	AccuBlue® NextGen dsDNA Quantitation Solution	31061, 31061-T	1000, 200 assays
Ultra High Sensitivity	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards	31028	1000 Assays
	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard	31029	4000 Assays
0.03-250 ng	AccuClear® Ultra High Sensitivity dsDNA Quantitation Solution	31027, 31027-T	1000, 200 assays
High Sensitivity	AccuBlue® High Sensitivity dsDNA Quantitation Kit with DNA Standard, Trial Size	31006-T	200 Assays
	AccuBlue® High Sensitivity dsDNA Quantitation Kit with 8 DNA Standards	31006	1000 Assays
0.2-100 ng	AccuBlue® High Sensitivity dsDNA Quantitation Solution	31008, 31008-T	1000, 200 assays
Broad Range	AccuBlue® Broad Range dsDNA Quantitation Kit with DNA Standard, Trial Size	31007-T	200 Assays
	AccuBlue® Broad Range dsDNA Quantitation Kit with 9 DNA Standards	31007	1000 Assays
2-2000 ng	AccuBlue® Broad Range dsDNA Quantitation Solution	31009, 31009-T	1000, 200 assays
High Sensitivity (for Qubit®) 0.1-100 ng	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)	31066, 31066-T	500, 100 assays
	AccuGreen™ High Sensitivity dsDNA Quantitation Solution (for Qubit®)	31068, 31068-T	500, 100 assays
Broad Range (for Qubit®) 2-1000 ng	AccuGreen™ Broad Range dsDNA Quantitation Kit (for Qubit®)	31069, 31069-T	500, 100 assays
	AccuGreen™ Broad Range dsDNA Quantitation Solution (for Qubit®)	31070, 31070-T	500, 100 assays
Stand-alone dsDNA standard sets	AccuBlue® Broad Range dsDNA Standards, Set of Nine (0-200 ng/uL calf thymus dsDNA)	31007C	0.5 mL each
	AccuBlue® High Sensitivity dsDNA Standards, Set of Eight (0-10 ng/uL calf thymus dsDNA)	31006C	0.5 mL each

AccuBlue and AccuClear are registered trademarks of Biotium, Inc. AccuBlue, AccuClear and AccuGreen technologies are covered by granted U.S. and international patents. Qubit is a registered trademark of Thermo Fisher Scientific.

GelRed® and GelGreen® Nucleic Acid Gel Stains

The safest and most sensitive gel stains

GelRed® and GelGreen® fluorescent nucleic acid gel stains were designed to replace highly toxic ethidium bromide (EtBr) and other so-called safe gel stains. To make safer gel stains, scientists at Biotium designed GelRed® and GelGreen® to make them cell membrane impermeable. 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, and thus these stains are classified as non-hazardous waste, permitting disposal down the drain or in regular trash.

For more information and references download our white paper, *Comparison of Nucleic Acid Gel Stains: Cell Permeability, Safety, and Sensitivity* and the complete *Safety Report of GelRed® and GelGreen®* at www.biotium.com.

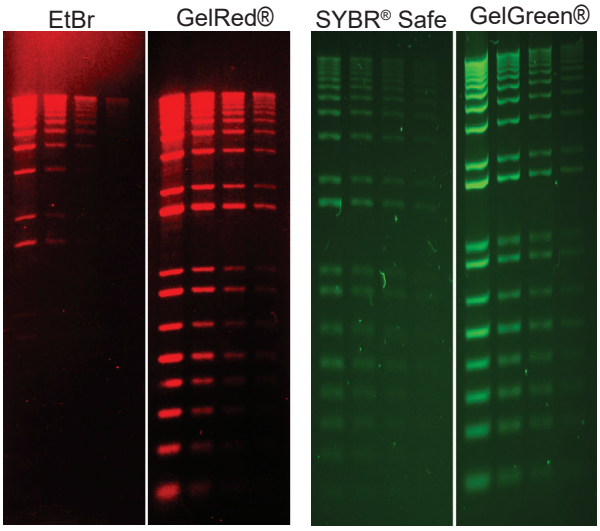


Figure 1. GelRed® and GelGreen® are more sensitive than EtBr and SYBR® Safe. Left: Comparison of GelRed® and ethidium bromide (EtBr) in precast gel staining using 1% agarose gel in TBE buffer. Right: Comparison of GelGreen® and SYBR® Safe in post gel staining using 1% agarose gel in TBE buffer. Serial dilutions of 1 kb Plus DNA Ladder from Invitrogen were loaded onto each gel: 200 ng, 100 ng, 50 ng and 25 ng, from left to right.

GelRed® and GelGreen® Features

- Safer than EtBr and other so-called safe gel stains.
- Passed environmental safety tests for direct disposal down the drain or in regular trash
- More sensitive than EtBr, SYBR® Safe, EZ-Vision® In-Gel Dye, and others
- Stable in solution at room temperature
- For precast or post-electrophoresis gel staining
- Compatible with standard instruments
- Compatible with downstream applications

Ordering Information

Cat. #	Product Name	Size
41003-T	GelRed® Nucleic Acid Gel Stain; 10,000X in water	0.1 mL
41003		0.5 mL
41003-1		10 mL
41002	GelRed® Nucleic Acid Gel Stain; 10,000X in DMSO	0.5 mL
41002-1		10 mL
41001	GelRed® Nucleic Acid Gel Stain; 3X in water	4 L
41005-T	GelGreen® Nucleic Acid Gel Stain; 10,000X in water	0.1 mL
41005		0.5 mL
41005-1		10 mL
41004	GelGreen® Nucleic Acid Gel Stain; 10,000X in DMSO	0.5 mL
41009	6X GelRed® Prestain Loading Buffer, Blue Tracking Dye	1 mL
41010	6X GelRed® Prestain Loading Buffer, Orange Tracking Dye	1 mL
31022	Ready-To-Use 1 kb DNA Ladder	150 lanes
31032	Ready-To-Use 100 bp DNA Ladder	150 lanes
31039	1 kb DNA Ladder	30 ug
31040	100 bp DNA Ladder	30 ug
22007	Activated Charcoal Decontamination Bags	25 bags
41006	5X TBE	4 L
31030-50	DNA Gel Extraction Kit	50 extrac-tions

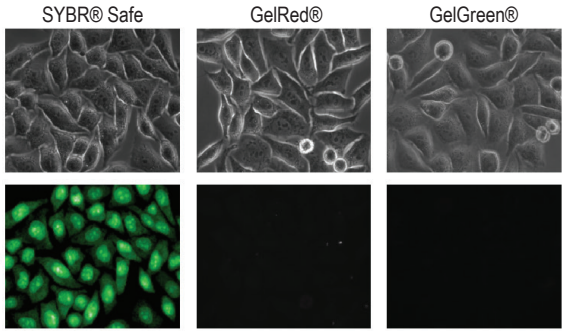


Figure 2. GelRed® and GelGreen® gel stains are safer because they cannot penetrate cell membranes to bind DNA in living cells. HeLa cells were incubated at 37°C with 1X SYBR® Safe, GelGreen® or GelRed®, respectively. Images were taken following incubation with dye for 30 min. The top row shows phase 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.

GelRed, GelGreen, and their uses are covered by granted and/or pending US and/or international patents. SYBR is a registered trademark of Thermo Fisher Scientific. EZ-Vision is a registered trademark of AMRESCO.

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The safety and sensitivity of GelRed® now for PAGE gels

Biotium scientists recognize that a fundamental approach for making a gel stain safe is to eliminate or minimize the chance for the dye to interact with genomic DNA in living cells. Based on this design principle, chemists at Biotium incorporated structural features to make the dyes impermeable to latex gloves, nitrile gloves, and cell membranes.

In the design of the original GelRed® and GelGreen® dyes, we achieved the dyes' membrane impermeability mainly by making the dyes physically large. While this strategy works extremely well to improve the dyes' safety and at the same time produces exceptional gel staining sensitivity for agarose gels, the relatively large size of GelRed® and GelGreen® make the dyes difficult to penetrate into the more densely packed polyacrylamide gels, rendering the dyes less optimal for PAGE gel staining. In designing PAGE GelRed® dye, we used a novel approach to make the dye membrane impermeable without making the dye large. Importantly, the new design strategy still ensures that the PAGE dye possesses essential properties for gel staining, including good sensitivity, stability and compatibility with existing instruments and downstream sample analysis.

A safer gel stain designed for use in acrylamide gels

- Formulated in water
- Impermeable to latex and nitrile gloves
- Non-toxic and non-mutagenic in AMES test
- Non-toxic to aquatic life, okay for drain disposal by EPA Title 22 hazardous waste test

Download the PAGE GelRed® Safety Report at www.biotium.com

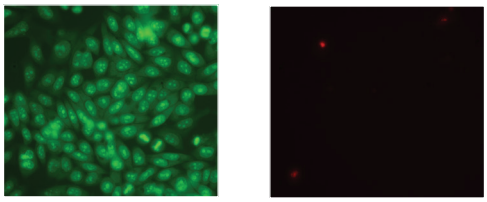


Figure 2. PAGE GelRed® is safer because it cannot penetrate cell membranes to bind DNA in living cells. HeLa cells were incubated at 37°C with 1X SYBR® Safe or 1X PAGE GelRed®. Images were taken following incubation with dye for 30 min using FITC filter set for SYBR® Safe and Cy®3 filter set for PAGE GelRed®. SYBR® Safe rapidly penetrated cell membranes as evident from the bright green staining of nuclei and cytoplasm. However, PAGE GelRed® was unable to cross cell membranes, as shown by the absence of fluorescence staining in healthy cells.

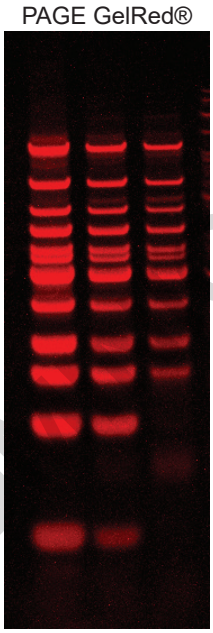


Figure 1. NEB low molecular weight ladder was separated on a 10% acylamide TBE gel (left to right, 500, 200, 100 ng/lane) and stained with 1X PAGE GelRed™ in water for 30 minutes. The gel was imaged on a UV transilluminator using a UVP GelDoc-It imaging system with ethidium bromide filter.

Ordering Information

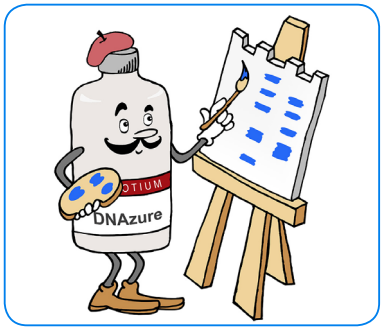
Cat. #	Product Name	Size
41008-T	PAGE GelRed® Nucleic Acid Gel Stain; 10,000X in water	0.1 mL
41008-500uL	PAGE GelRed® Nucleic Acid Gel Stain; 10,000X in water	0.5 mL
41014	PAGE GelRed® Nucleic Acid Gel Stain; 1X in water	4 L

DOCUMENTATION TECHNIQUE DUTSCHER SAS

PAGE GelRed and its uses are covered by granted and/or pending US and/or international patents. SYBR is a registered trademark of Thermo Fisher Scientific. Cy is a registered trademark of GE Healthcare.

DNAzure® DNA Gel Stain

Visualize DNA bands in gels by eye, with sensitivity rivaling the best fluorescent gel stains



DNAzure® Blue Nucleic Acid Gel Stain is an ultrasensitive reagent for visible staining of dsDNA in agarose gels or polyacrylamide gels. The sensitivity of this stain is comparable to fluorescent DNA gel stains. The limit of detection is 1 ng dsDNA or less.

Key to the technology is a DNA-binding dye that turns from colorless to deep blue upon exposure to bright light. Light exposure can be performed with a variety of different white or blue light sources. For best results, we recommend performing the light exposure with either the Gel-Bright™ LED Gel Illuminator or the Glo-Plate™ Blue LED Illuminator (see p. 13).

DNAzure® Features

- Deep blue bands visible by the naked eye after exposure to bright light
- Ultrasensitive detection, as little as ~1 ng DNA
- Simplified DNA band excision, without the need for DNA damaging UV light
- Compatible with downstream applications such as sequencing and cloning
- Expensive gel documentation systems not required for imaging
- Gels can be dried for long term storage

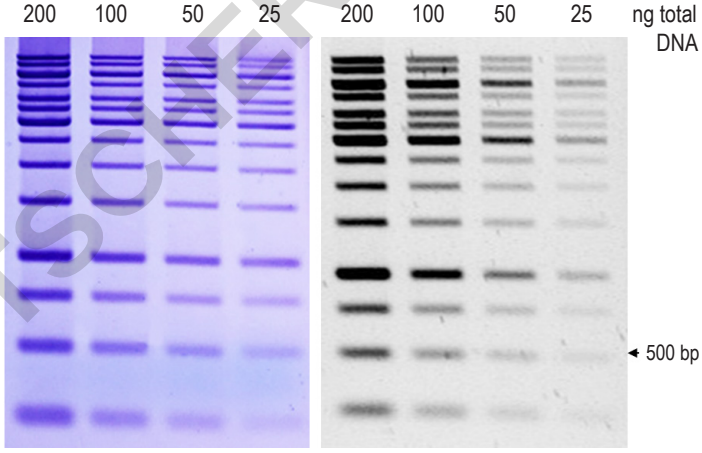


Figure 1. Biotium's 1 kb DNA ladder was loaded on a 1% agarose gel in two-fold dilutions. The gel on the left was stained with DNAzure® Blue Nucleic Acid Gel Stain for 25 minutes, and then the visible blue DNA bands were developed for 30 minutes using the Glo-Plate™ Blue LED transilluminator. The gel was imaged with a cell phone camera. The gel on the right was stained with 3X GelRed® Nucleic Acid Gel Stain for 60 minutes. The gel was imaged with a UVP GelDoc-It™ Imaging System using a UV transilluminator and EtBr filter.

Gel-Bright™ LED Gel Illuminator

The Gel-Bright™ LED Gel Illuminator is a light-weight, non-UV lightbox for detection of fluorescently labeled nucleic acids and proteins. This instrument possesses novel features enabling imaging performance superior to that of other LED illuminators on the market. The proprietary light source and unique filter mechanism ensure optimal excitation and clear visualization of both green and red fluorescent dyes.



- Ideal light source for visualization of gels stained with GelGreen® or GelRed®
- Excellent light source for development of blue bands in DNAzure®-stained gels
- SAFE! Avoid using DNA-damaging UV light sources
- Perfect for band excision

Ordering Information

Cat. #	Product Name	Size
41020	DNAzure® Blue Nucleic Acid Gel Stain, 100X	10 mL
E90003	Gel-Bright™ LED Gel Illuminator	1 instrument

DOMINIQUE DUTSCHER SAS

One-Step Protein Gel Stains

One-Step protein gel stains are ready-to-use solutions for the staining of PAGE gels. They produce fast protein staining in a single step without fixation or washing. In addition to rapid results and simple staining, One-Step stains offer safer handling and disposal compared to Coomassie and other stains because they are entirely aqueous-based, without hazardous methanol or acetic acid.

Biotium offers several versions of One-Step stains for different visualization methods. With One-Step Blue®, proteins can be detected by visible blue staining, or by near-infrared fluorescence. One-Step Lumitein™ Protein Gel Stain is a red fluorescent protein gel stain that is able to be detected using a UV light box, laser gel scanner or blue light illuminator like Gel-Bright™. And One-Step Lumitein™ UV is optimized for maximum sensitivity on a UV transilluminator.

One-Step Blue®

- Faster and simpler than tedious Coomassie staining for visible blue imaging of bands
- Optional near-infrared fluorescence detection with NIR imaging systems (such as Odyssey®)
- Lower limit of detection ~ 10-20 ng of protein

One-Step Lumitein™

- Replaces time-consuming and expensive SYPRO® Ruby gel stain for fluorescence-based imaging
- Detect with a laser-based fluorescence scanner (such as Typhoon®) or a UV gel box

One-Step Lumitein™ UV

- Unlike Oriole® fluorescent gel stain, does not cause gel shrinkage
- Best dye for detection with a UV gel box
- Detect as little as 1-10 ng of protein

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One-Step Blue®

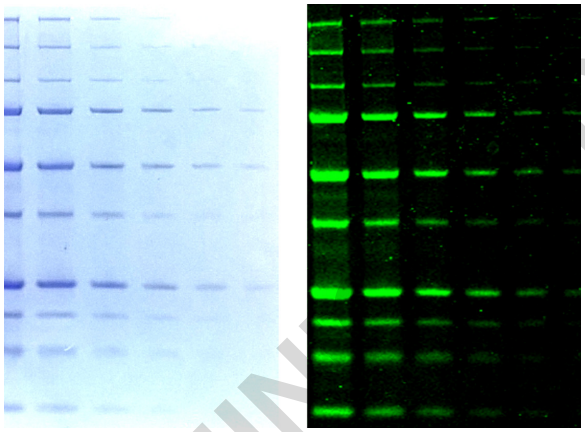


Figure 1. A protein ladder was loaded onto an SDS PAGE gel in 2-fold dilutions, and the gel was stained with One-Step Blue®. One-Step Blue® staining can be visualized as visible blue bands (left), or NIR fluorescence (right).

One-Step Lumitein™

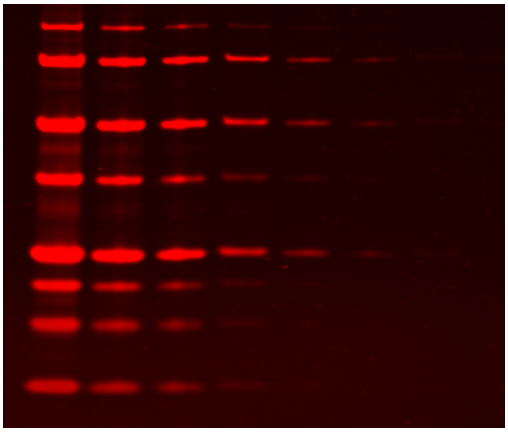


Figure 2. A protein ladder was loaded onto an SDS PAGE gel in 2-fold dilutions, and the gel was stained with One-Step Lumitein™. One-Step Lumitein™ fluorescent staining can be visualized on the Typhoon® or with a UV gel box.

One-Step Lumitein™ UV vs Oriole®

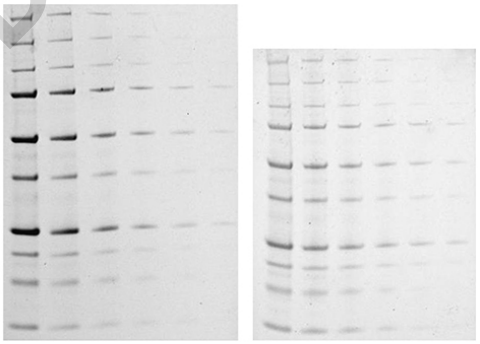


Figure 3. Identical SDS-PAGE gels were stained with One-Step Lumitein™ UV (left), or Oriole® (right). The gels began as the same size, but staining with Oriole® shrinks the gel.

Ordering Information

Cat. #	Product Name	Size
21003-1L	One-Step Blue® Protein Gel Stain	1 L
21004-1L	One-Step Lumitein™ Protein Gel Stain	1 L
21004-4L		4 L
21005-1 L	One-Step Lumitein™ UV Protein Gel Stain	1 L
21005-4L		4 L

One-Step Blue and One-Step Lumitein are trademarks of Biotium.
Oriole is a registered trademark of Bio-Rad Laboratories; Odyssey is a registered trademark of LI-COR Inc.; Typhoon is a registered trademark of GE Healthcare.

PMAxx™ and PMA for viability PCR

Selectively amplify DNA from live cells

Viability PCR (v-PCR)

Viability PCR is a powerful technology for the sensitive and rapid detection of viable microorganisms. Unlike time-consuming culturing methods, qPCR is a fast and sensitive method of detection. However, normal PCR does not distinguish between live and dead cells. With v-PCR using PMAxx™ or PMA, you get the speed, sensitivity and specificity of PCR, plus quantifiable viability. And because no culturing is required, you can even detect viable but not culturable (VBNC) bacteria.

How does v-PCR work?

PMAxx™ and PMA are photoreactive dyes with high affinity for DNA. The dyes intercalate into dsDNA and form a covalent linkage upon exposure to intense visible light (Figure 1). PMAxx™ and PMA inhibit PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases. Because PMAxx™ and PMA are cell membrane-impermeable, when a sample containing both live and dead bacteria is treated with dye, only dead bacteria with compromised cell membranes are susceptible to DNA modification. In a real-time PCR reaction, dead cell DNA will show delayed amplification and higher Ct than live cells (Figures 2 & 3). In a mixed population, v-PCR permits quantitation of cell viability. The v-PCR technology can be applied not only to bacteria but to other cell types as well.

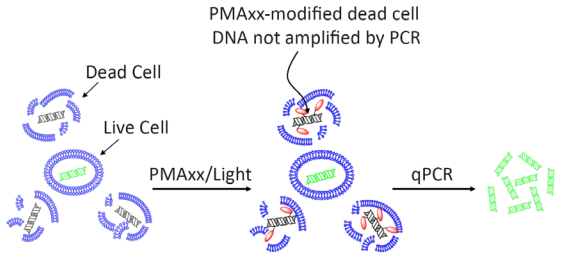


Figure 1. The cell membrane-impermeable PMAxx™ dye selectively and covalently modifies DNA from dead bacteria with compromised membrane while leaving DNA from viable cells intact. Because PMAxx™-modified DNA can not be amplified, subsequent lysis of viable cells and qPCR permit selective quantitation of viable bacteria. The same mechanism applies to PMA.

Viability PCR Uses

- Used in bacteria, yeast and some viruses
- Food safety
- Environmental testing
- Infectious disease testing
- Probiotic detection
- Microbiology research

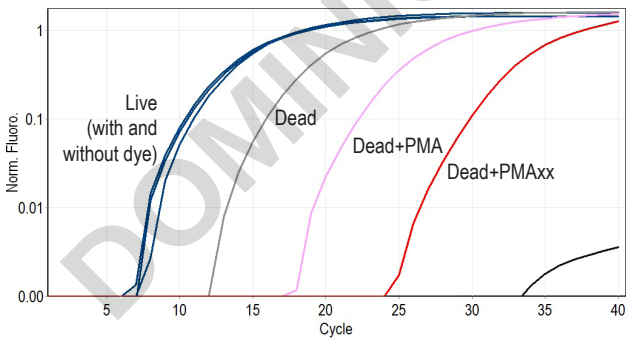


Figure 2. Comparison between PMAxx™ and PMA in viability PCR. Living and heat-killed Bacillus subtilis bacteria were treated with 25 uM PMAxx™, 25 uM PMA, or left untreated. After photolysis and DNA isolation, qPCR was performed using primers against the Bacillus subtilis gyrA gene. Shown are the normalized amplification curves for the real-time PCR reaction performed with DNA from PMAxx™- and PMA-treated live and heat-killed Bacillus subtilis.

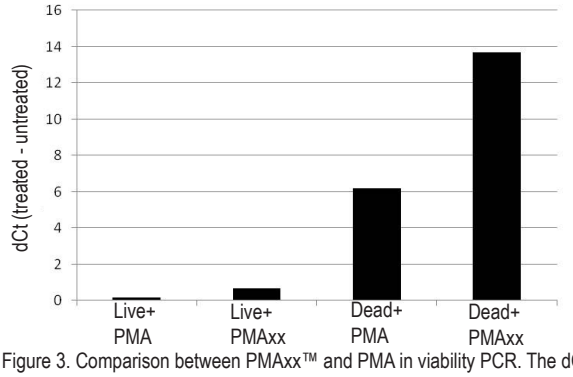


Figure 3. Comparison between PMAxx™ and PMA in viability PCR. The dCt of live and killed Bacillus subtilis with PMAxx™ or PMA treatment. The Ct value of untreated sample was subtracted from the corresponding sample treated with PMAxx™ or PMA (dCt = Ct with dye – Ct without dye).

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PMAxx is a trademark of Biotium. PMAxx and its uses are covered by granted or pending US and/or international patents.

PMA Real-Time PCR Bacterial Viability Kits

v-PCR kits are designed for selective detection of viable bacteria from a specific strain using PMAxx™ or PMA dye and real-time PCR. We offer kits for detection of selected strains of bacteria that are of widespread interest to food safety, public health, and/or antibacterial research. More strains are being added, so check our website for current offerings.

Kits include:

- PMAxx™ or PMA dye
- Forget-Me-Not™ qPCR Master Mix
- ROX reference dye
- Validated PCR primers for specific bacterial strain
- 5X PMA Enhancer for Gram-Negative Bacteria (gram-negative strain kits only)

Kits available for:

- Salmonella enterica
- Staphylococcus aureus
- MRSA
- Escherichia coli and Escherichia coli O157:H7
- Mycobacterium tuberculosis
- Listeria monocytogenes
- Legionella pneumophila

LED Photolysis Devices

PMA-Lite™

The PMA-Lite™ LED Photolysis Device is designed for photoactivation of PMAxx™- and PMA-treated samples in microcentrifuge tubes.

- Provides even illumination to up to 18 microcentrifuge tubes
- Internal fan to ensure a temperature of <37^{O}C
- Four timer settings for 10, 15, 20 or 30 minutes of illumination



Glo-Plate™ Blue

The Glo-Plate™ Blue LED Illuminator is designed for photoactivation of PMAxx™- and PMA-treated samples in microplate or other assay format.

- Provides even illumination across a flat surface
- Surface stays cool to protect your samples
- Four timer settings for 10, 15, 20 or 30 minutes of illumination



PMA Enhancer for Gram-Negative Bacteria

Under some conditions such as mild heat treatment, bacteria may be dead but retain intact membranes that have lower permeability to PMAxx™ or PMA than those of boiled bacteria, for example. This could result in an overestimate of live bacteria. Biotium has developed an Enhancer for use with Gram-Negative bacteria that can greatly improve live/dead discrimination (Figure 4). The Enhancer works with both PMAxx™ and PMA.

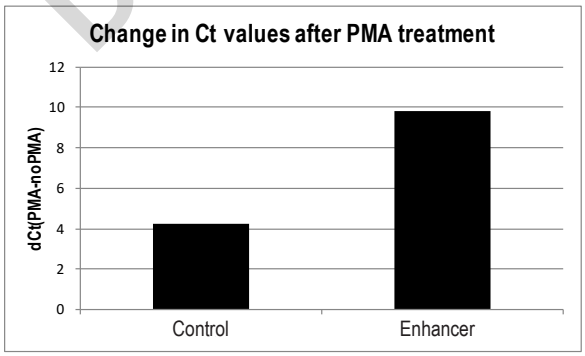


Figure 4. PMA plus Enhancer for quantitation of viable bacteria by Real-time PCR. Mildly heat-killed E. coli were treated with PMA +/- Enhancer, followed by exposure with the PMA-Lite and DNA purification. dCt values were calculated by subtracting the Ct without PMA from the Ct with PMA. For dead cells, the use of Enhancer increased the dCt from 4 to 10, greatly increasing the specificity of viability PCR.

Ordering Information

Cat. #	Product Name	Size
40069	PMAxx™ Dye, 20 mM in dH_2O	100 uL
40013	PMA Dye	1 mg
40019	PMA Dye, 20 mM in dH_2O	100 uL
E90004	Glo-Plate™ Blue LED Illuminator	1 device
E90002	PMA-Lite™ LED Photolysis Device	1 device
31038	PMA Enhancer for Gram-Negative Bacteria	16 mL
31033	PMA-PCR Bacterial Viability Kit-Salmonella	200 assays
31034	PMA-PCR Bacterial Viability Kit-M. tuberculosisis	200 assays
31035	PMA-PCR Bacterial Viability Kit-Staph. aureus	200 assays
31036	PMA-PCR Bacterial Viability Kit-MRSA	200 assays
31037	PMA-PCR Bacterial Viability Kit-E. coli O157:H7	200 assays
31050	PMA-PCR Bacterial Viability Kit-E. coli	200 assays
31051	PMA-PCR Bacterial Viability Kit-Listeria	200 assays
31053	PMA-PCR Bacterial Viability Kit-Legionella	200 assays

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DNA purification kits

RNAstorm™ and DNAstorm™ FFPE extraction kits with CAT5™ technology

Higher quality nucleic acids
Most existing methods rely on heat to remove crosslinks and adducts, which is only partially effective and leads to additional fragmentation of labile nucleic acids. In contrast, the catalytic CAT5™ technology included in the RNAstorm™ and DNAstorm™ kits greatly accelerates the removal of formaldehyde damage and allows recovery of higher quality nucleic acids.

Best choice for NGS
NGS library preparation relies on multiple enzymatic steps, including reverse transcription and amplification, which require nucleic acids with high integrity and amplifiability. The RNAstorm™ and DNAstorm™ kits provide a sample processing solution specifically tailored for advanced downstream applications, and which allows end-users to reliably work with challenging yet valuable formalin-fixed samples.

Convenient workflow
RNAstorm™ and DNAstorm™ nucleic acid extraction kits offer a convenient and efficient protocol for purification of high quality RNA and DNA.

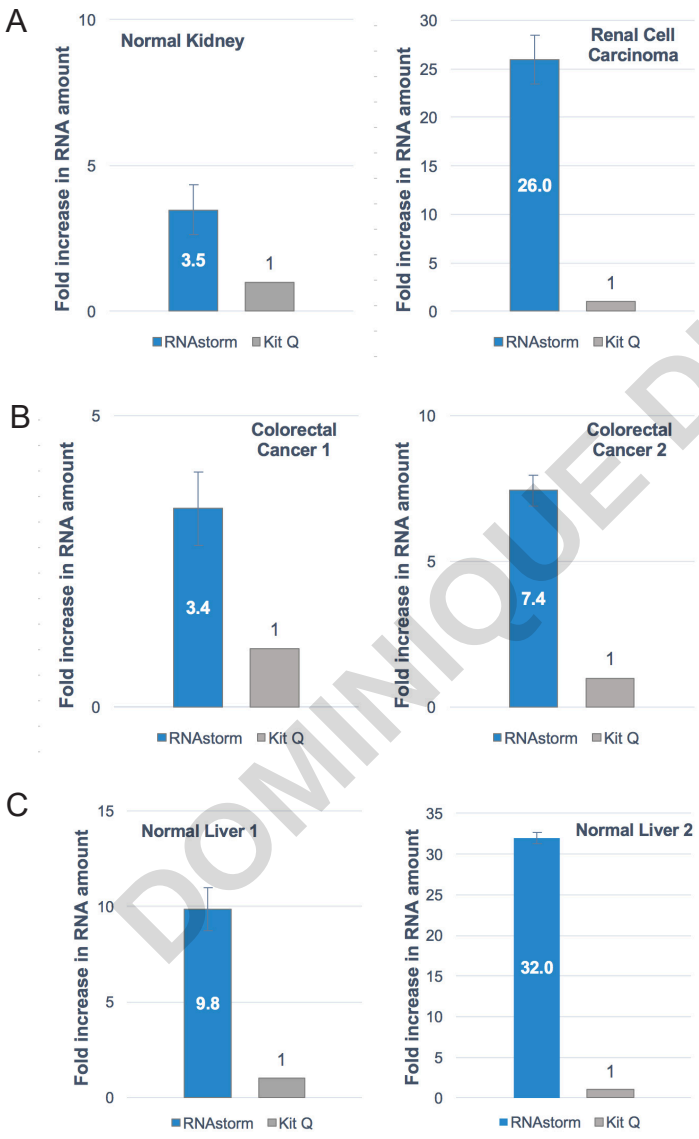


Figure 1. Comparison of RNA recovery by quantitative RT-PCR from FFPE tissues. "Q" represents a competitor commercial FFPE RNA extraction kit. Significant improvements in RNA yield and quality (as measured by amount of amplifiable RNA) are seen using a prototype of the RNAstorm™ kit on FFPE samples from various tissues and ranging in age from 1976 to 2015.

RNAstorm™ advantages

- Higher yields of amplifiable RNA, 3-30 fold more than other kits
- Higher integrity RNA: more RNA > 200 nt, critical for successful NGS
- Simple and convenient workflow

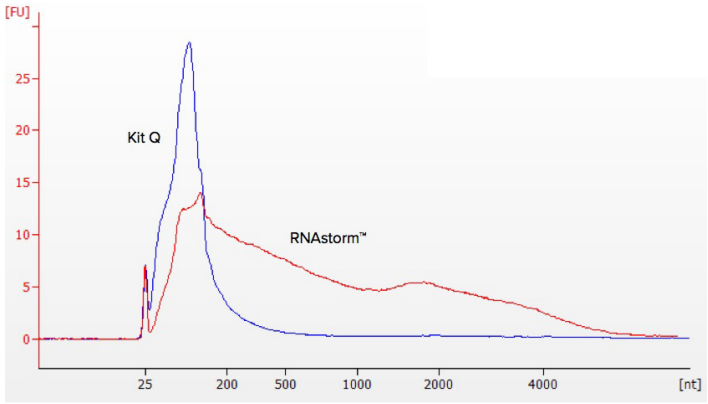


Figure 2. Increased RNA integrity is observed for RNA extracted using the RNAstorm™ kit. An exemplary comparison with a popular commercial kit is shown below (Agilent Bioanalyzer RNA 6000 nano).

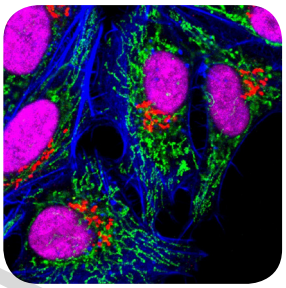
Ordering Information

Cat. #	Product Name	Size
31030-250	DNA Gel Extraction Kit	250 reactions
31030-50		50 reactions
CD501	RNAstorm™ FFPE RNA Extraction Kit	50 reactions
CD201		20 reaction
CD502	DNAstorm™ FFPE RNA Extraction Kit	50 reactions
CD202		20 reaction

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CF® dyes and related products

CF® dyes are next-generation fluorescent dyes with unbeatable brightness and photostability.

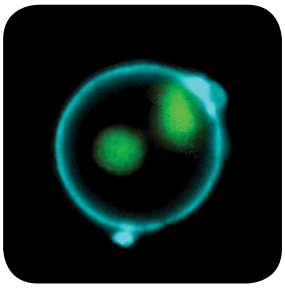


CF® dye products:

- Dye-conjugated primary and secondary antibodies
- Single-labeled secondary antibodies for STORM
- Reactive dyes for labeling biomolecules
- Simple Mix-n-Stain™ kits for rapid antibody or small molecule labeling
- Other dye-conjugated biomolecules such as nucleotides, lectins, and phalloidin

Cell viability and apoptosis assays

Viability assays and reagents to monitor apoptosis, cell viability, and membrane potential. Assays for microscopy, flow cytometry and plate reader.



Cell viability products:

- NucView® Caspase 3 Enzyme Substrates
- Fluorescently-labeled Annexin V (apoptosis marker)
- CF® dye TUNEL assay kits (apoptosis)
- Live-or-Dye™ Fixable Viability Staining Kits
- ViaFluor® Cell Proliferation kits for flow cytometry
- MitoView™ mitochondrial membrane potential dyes

Luciferase assays

Our new, improved luciferase assays have excellent stability, sensitivity and linearity.

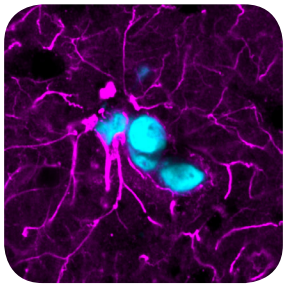


Luciferase products:

- Firefly Luciferase Assay 2.0
- Renilla Luciferase Assay 2.0
- Firefly and Renilla Single Tube Assay
- Steady-Luc™ Firefly HTS Assay
- ATP-Glo™ Luminescent Cell Viability Assay
- Luciferin and coelenterazine luciferase substrates

Accessories and other products

Biotium offers many other useful technologies and accessory products to improve your experiments.



Other products:

- TrueBlack® autofluorescence quencher
- EverBrite™ mounting media, CoverGrip™ coverslip sealant and other microscopy products
- DNA ladders, dNTPs, and other molecular biology accessory products
- Mini cell scrapers, fixation/permeabilization reagents, and other flow cytometry products

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Biotium