Product Information

PMAxx™ Dye, 20 mM in H_2O

Product List

Cat. No.	Unit Size
40069	100 uL
40069-1ML	1 mL

Storage and Handling

Store PMAxx™ Dye at -20°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Absorption/Emission:

464 nm (before photolysis)
510/610 nm (after photolysis and reaction with DNA/RNA)

Product Description

PMAxx™ Dye is a high-affinity photoreactive DNA binding dye invented by scientists at Biotium for viability PCR (v-PCR) of bacteria and other organisms. In v-PCR, pretreatment of a cell culture with a viability dye, such as PMAxx™ or PMA, allows differentiation of live and dead cells using qPCR or other DNA amplification methods. It has been validated in a wide variety of bacterial strains, as well as yeast and viruses.

PMAxx™ binds to dsDNA with high affinity. Upon photolysis, the dye covalently reacts with DNA, resulting in permanent DNA modification (Figure 1). PMAxx™ is cell membrane-impermeant, and can be used to selectively modify only the DNA in dead cells while leaving the DNA in viable cells intact. PMAxx™ inhibits PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases (1). This feature makes the dye highly useful in the selective detection of viable cells by qPCR.

In experiments with laboratory bacterial strains, PMAxx™ increases the difference between live and dead cells a further 3-7 Ct compared to PMA. Therefore, v-PCR with PMAxx™ is more effective at discriminating between live and dead bacteria. Because PMAxx™ works the same way as PMA, it can directly replace PMA in your current v-PCR protocol.

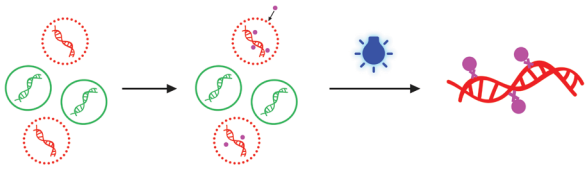

Figure 1. Principle of PMAxx™ modification of dead cell DNA. The cell membrane-impermeant PMAxx™ Dye (purple dot) selectively enters dead cells with compromised membranes (red) and after light treatment, covalently modifies the DNA. Subsequent PCR amplification of PMAxx™-modified DNA templates is inhibited, allowing selective quantitation of DNA from viable cells (green).

Considerations for Viability PCR

1. v-PCR differentiates viable from non-viable cells based on cell membrane permeability. Many methods of killing cells result in compromised cell membranes, and are thus compatible with v-PCR. However, some methods, such as UV light exposure, may not immediately result in disrupted cell membranes. Literature searches and pilot studies may help to determine whether v-PCR will work with your chosen cell type and killing method.
2. It is advisable to choose the appropriate dye for your v-PCR experiment. Biotium offers three different v-PCR dyes: EMA, PMA, and PMAxx™. In general, we recommend using PMAxx™ for bacterial samples and PMA for yeast and fungal samples. EMA is more permeant to live cells and thus often gives false negative results. However, you may want to test more than one dye to determine the optimal dye for your sample type.
3. Primer selection is an important consideration. If you would like to simultaneously detect all species of bacteria in a mixture, primers against rRNA targets that are known to be pan-species-specific are a good choice. If detection of only one species or strain of bacteria is desired, you will want to design or find specific primers. Dye molecules will be bound randomly along the DNA strand. Therefore, the longer the amplicon, the more likely it will be that a dye molecule will be bound in that region. It is recommended that an amplicon of at least 100 bp is used, and longer amplicons generally give better results.
4. Freezing samples prior to performing v-PCR may damage the cell membrane and give false negative results. We have found that freezing affects gram-positive bacteria more than gram-negative bacteria. A pilot test of your sample of interest is recommend before attempting pre-dye-treatment freezing. After the photolysis step, samples can be frozen.

5. If your sample of interest is a gram-negative bacterium, you may want to use PMA Enhancer for Gram Negative Bacteria (Cat. No. 31038). When this product is added to the sample before PMAxx™ treatment, dead cell DNA levels are further decreased and live/dead cell discrimination is improved. However, if your sample is a gram-positive strain, or a mixture of gram-negative and gram-positive, the Enhancer should not be used.
6. v-PCR requires a photoactivation step in order for the dye to covalently bind to the dead cell DNA. Biotium offers the PMA-Lite™ 2.0 LED Photolysis Device (Cat. No. E90006) for use with 1.5 mL tubes and the Glo-Plate™ 2.0 Blue (Cat. No. E90007) for use with clear microplates. Other blue or white light sources may also be used. In general, the brighter the lights, the more efficiently they will perform the photolysis step. Non-LED lights, such as halogen lamps, may heat your sample and negatively affect the assay.
7. Part of the proposed mechanism of action of PMAxx™ is the removal of PMAxx™-bound DNA from samples via precipitation. Therefore, the amount of template DNA in each qPCR reaction should not be normalized between samples. Instead, PCR should be performed using equal volumes of gDNA eluate from each sample. As a positive control for the qPCR reaction, 1 ng of purified genomic DNA (gDNA) should be sufficient for achieving good signal.
8. In order to validate PMAxx™ effectiveness in your sample of interest, it is best to perform live cell and dead cell controls, each with and without PMAxx™ (Figure 2). The change in Ct (dCt) caused by PMAxx™ for each control should be assessed (see Data Analysis).

Before You Begin

- Read the Considerations for Viability PCR section to determine the appropriate viability dye, primers, and light source to use in your experiment.
- Ensure that you have a workspace that is protected from direct light. The steps of the protocol that require opening the vial of PMAxx™, pipetting PMAxx™, and incubating with PMAxx™ should be done with lights dimmed.

Experimental Protocols

1. Standard v-PCR protocol

See Figure 2 for an overview of the procedure. This is a general protocol for treating cultured strains of bacteria with PMAxx™. It is recommended that you first perform a control experiment with live and dead controls for your selected organism. Treatment of complex samples, such as feces or soil, may require optimization of sample dilution, dye concentration, and light treatment time. Treatment of dilute samples, such as water testing, may require filtration or concentration before PMAxx™ treatment.

1.1 Inoculate your desired media broth with bacteria (volume is dependent on size of experiment). Culture bacteria overnight or longer until the OD600 of the culture is approximately 1.

1.2 (Suggested): To prepare dead cell control samples, heat-inactivate bacteria at 90°C for 5 minutes. If it is desired to compare v-PCR with plate-based viability, plate 10 uL of heat-inactivated bacteria on the appropriate media plate and 10 uL of a 1:100 dilution of control bacteria on another plate. Grow on plates at optimal growth temperature and check for colony growth.

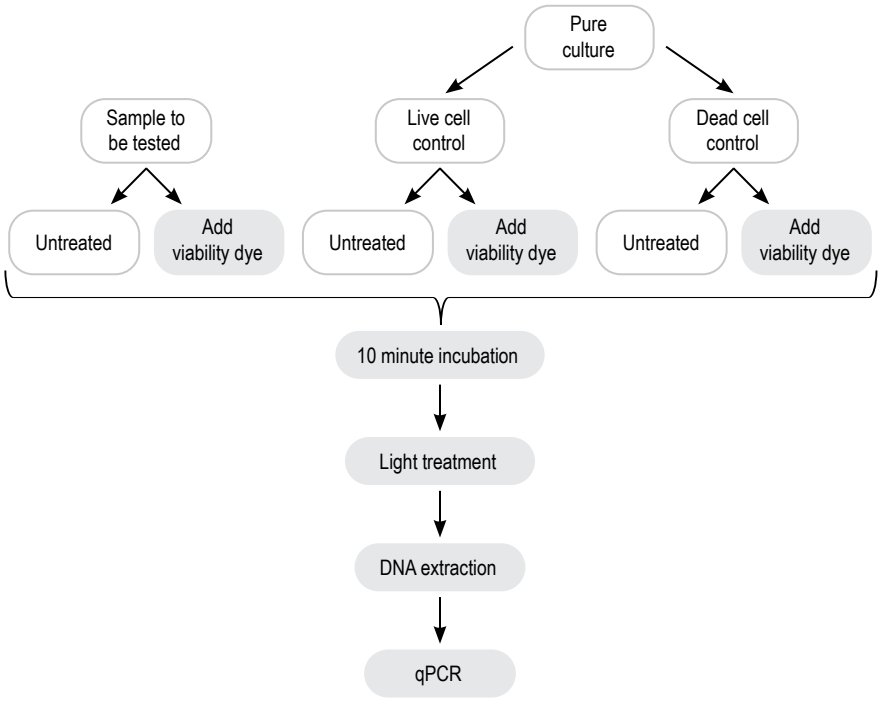

Figure 2. v-PCR workflow overview, with recommended live and dead cell controls.

1.3 Pipette 400 uL aliquots of bacterial culture into clear microcentrifuge tubes. For each sample you will need one tube for PMAxx™-treated cells and one tube for untreated (no dye added) in order to calculate dCt (see Figure 2, Considerations for Viability PCR #8, and Data Analysis for details).

1.4 (Optional, gram-negative bacteria only): Add 100 uL of PMA Enhancer for Gram Negative Bacteria, 5X (Cat. No. 31038), for a 1X final concentration.

1.5 Working in low light, add the appropriate volume of PMAxx™ stock for a final concentration of 25 uM (e.g., 1 uL of 10 mM stock in 400 uL).

1.6 Incubate tubes in the dark for 10 minutes at room temperature. Perform incubation on a rocker for optimal mixing.

1.7 Expose samples to light to cross-link PMAxx™ to DNA. 15 minutes in the PMA-Lite™ is a good starting point for most samples, but complex or opaque samples may need a longer exposure time. See Considerations for Viability PCR #6 for information on light sources.

1.8 Pellet cells by centrifuging at 5,000 x g for 10 minutes.

1.9 Extract genomic DNA using your desired protocol or commercially available kit for your sample type.

1.10 Perform qPCR for each sample with the same volume of genomic DNA in each reaction, and do not normalize to ug of DNA. See Considerations for Viability PCR #7 for more information. Use primers against a chosen genomic DNA target for your organism of interest. See Considerations for Viability PCR #3 regarding primer selection.

1.11 (Optional): If it is desired to determine the absolute number of viable cells in your sample, you should also include, as templates in the qPCR reaction, genomic DNA from your cell type of known cell number (see Calculating the absolute number of viable cells in Data Analysis).

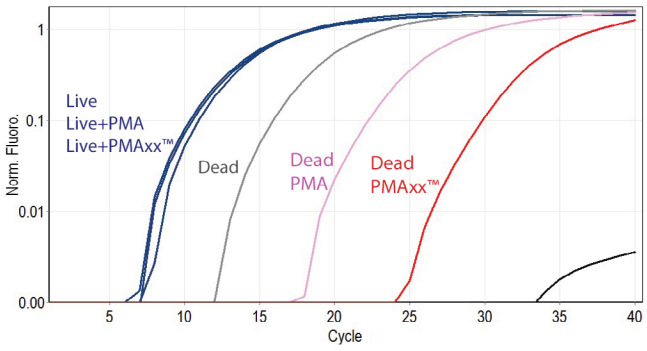

Figure 3. Normalized qPCR curves from a v-PCR experiment in which live and heat-inactivated *B. subtilis* were treated with PMA or PMAxx™. Neither PMA or PMAxx™ treatment had an effect on amplification of DNA from live *B. subtilis*, but caused a dramatic delay in amplification of DNA from heat-killed *B. subtilis.* PMAxx™ treatment showed a significant further delay compared to dead cells treated with PMA.

2. Data analysis

This section describes how to use the live and dead cell controls to determine whether your experiment worked, and how to calculate the percentage of live cells in your sample. It is advisable to validate your primers and PCR setup with genomic DNA from the same cell type before beginning your v-PCR experiment.

Live & dead cell control dCt determination

2.1 After the qPCR run, use the instrument software to determine the threshold cycle (Ct) for each of your samples.

2.2 In order to determine whether PMAxx™ adequately inhibited amplification of dead cell DNA, calculate the delta Ct (dCt) for each of your control cell populations as shown:

$$dCt_{live} = Ct_{(live,\ PMAxx\text{-}treated)} - Ct_{(live,\ untreated)}$$
$$dCt_{dead} = Ct_{(dead,\ PMAxx\text{-}treated)} - Ct_{(dead,\ untreated)}$$

Notes:

a. The expected result for the live cell control is a dCt close to 0 +/- 1 (Figure 4). This indicates that PMAxx™ treatment did not affect viable cell DNA amplification. If a larger dCt is seen for the live cell control, see Troubleshooting.

b. The expected result for the dead cell control is a dCt > 4 (Figure 4). Since Ct values are on a $\log_2$ scale, a dCt of 4 represents a ~ 16-fold decrease, or 94% of dead cell DNA removed. A dCt of 8 represents a ~250-fold decrease, or 99.6% of dead cell DNA removed. If a low dCt is seen for the dead cell control, see Troubleshooting.

c. The dead cell dCt that you obtain will depend on many factors, including the bacterial strain or other cell type, how the cells were killed, the concentration of viability dye used, the amplicon length, and whether Enhancer (Cat. No. 31038) was used. We have found that at 25 uM PMAxx™, the dCt ranges from ~9 to 13, depending on the bacterial strain. In yeast, we have obtained a dCt of 7 for 100 uM PMAxx™.

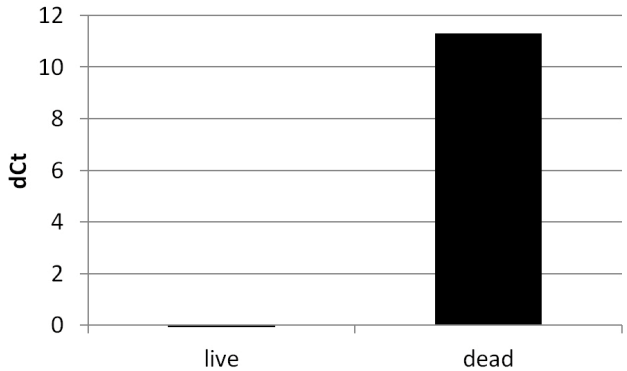

Figure 4. The difference in Ct (dCt; $Ct_{PMAxx\text{-}treated} - Ct_{untreated}$) of live and heat-killed *E. coli*. The dCt is used to evaluate the effectiveness of PMAxx™ in your samples. PMAxx™ has no effect on live cell DNA, thus the dCT is close to 0. Dead cells treated with PMAxx™ exhibit delayed amplification of DNA compared to untreated dead cells, which causes a large dCt.

Calculating the percentage of viable cells

If your live and dead cell controls look good, you can move on to determining the percentage of viable cells in your unknown samples.

2.3 Calculate the dCt for the unknown samples as shown:

$$dCt_{sample} = Ct_{(sample,\ PMAxx\text{-}treated)} - Ct_{(sample,\ untreated)}$$

2.4 You can convert the dCt into a percentage of viable cells as shown:

$$\text{Fold reduced by PMAxx™} = 2^{(sample\ dCt)}$$
$$\%\ \text{viable} = 100 / \text{Fold reduced}$$

Calculating the absolute number of viable cells

If you want to calculate the absolute number of viable cells in your sample, then in the same experiment you will need to run a standard curve using genomic DNA from your cell type of interest, from a known cell number. It is advisable that you have several gDNA dilutions which fall within the predetermined linear range of your qPCR assay.

Note: This assumes that none of the viable cell DNA has been lost during the DNA purification process.

2.5 Using the genomic DNA samples that fall within the linear range of the qPCR assay, plot a graph of Ct (y-axis) vs cell number (x-axis). Use graphing software to calculate the R^2 value (to determine linearity of the assay), slope, and y-intercept of the line.

2.6 Use the slope and y-intercept to calculate the copy number of your unknown sample as shown:

$$Ct = \text{slope}(\text{cell number}) + \text{y-intercept}$$
$$\text{Cell number} = (Ct - \text{y-intercept}) / \text{slope}$$

References

1. J Microbiol Methods 67, 310 (2006).

Troubleshooting

Problem	Solutions
No positive qPCR signals are seen in any sample above the No Template Control (NTC).	Optimize the qPCR reaction using purified genomic DNA from the same cell/strain type that you are using in your v-PCR experiment. Ensure that your chosen primers, master mix, and program work well. Increase the template volume, if necessary.
High dCt seen in the live cell control sample.	A high dCt value (i.e., > 1) in the live cell control sample generally indicates that the viability dye has penetrated through the live cell membrane. • Confirm that your cells are actually alive. Use a dead cell stain such at Ethidium Homodimer III (Cat. No. 40050) to measure the membrane integrity of your cells. • Ensure that you are not using the PMA Enhancer for Gram Negative Bacteria (Cat. No. 21038) with gram-positive bacteria. • Ensure that there is no detergent present in your samples. • Ensure that you are not freezing your samples prior to PMAxx™ treatment. • Try a lower dye concentration. Titrate the dye until you get an effective concentration that only alters dead cell DNA. • If you are treating your cells in a simple buffer (i.e., PBS) or water, try treating them in media, or buffer containing BSA or other blocking protein.
Low dCt seen in the dead cell control sample.	A low dCt value (i.e., < 4) in the dead cell control sample can be caused by many different factors. • Confirm that your cells are actually dead. Use a dead cell stain such at Ethidium Homodimer III (Cat. No. 40050) to measure the membrane integrity of your cells. See Considerations for Viability PCR #1 for more details. • Try a higher dye concentration. Titrate the dye until you get an effective concentration that only alters dead cell DNA. • If your cells are gram-negative bacteria, try using the PMA Enhancer for Gram Negative Bacteria (Cat. No. 31038). • If you are using the Glo-Plate™ 2.0 (Cat. No. E90007), increase the light exposure time (for example, 30 minutes instead of 15). • Ensure that the amplicon that you are amplifying is at least 100 bp. If possible, try using primers for a longer amplicon. • Be sure to use the same volume of eluted DNA in each PCR reaction and do not normalize to ug of DNA (see Considerations for Viability PCR #7 for additional details). • If your samples are complex (such as soil or feces), try diluting them in sterile PBS, increasing the light exposure with more frequent mixing, and increasing the PMAxx™ concentration up to 100-200 uM.

Related Products

Cat. No.	Product
40013, 40019	PMA (Propidium Monoazide)
40015	Ethidium Monoazide Bromide (EMA)
E90006	PMA-Lite™ 2.0 LED Photolysis Device
E90007	Glo-Plate™ 2.0 Blue LED Illuminator
31038	PMA Enhancer for Gram-Negative Bacteria, 5X Solution
31075, 31076	Viability PCR Starter Kits
31033... 31053	PMA Real-Time PCR Bacterial Viability Kits
40050	Ethidium Homodimer III (EthD-III)
40051	Ethidium Homodimer III (EthD-III), 1 mM in DMSO
32019, 32020	BactoView™ Viability Kits
40101, 40102	BactoView™ Live Fluorescent Bacterial Stains
40107-40113	BactoView™ Dead Stains
32000	Live Bacterial Gram Stain Kit
32001	Bacterial Viability and Gram Stain Kit
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells
32002... 32018	Live-or-Dye™ Fixable Viability Staining Kits
32010	Live-or-Dye™ NucFix™ Red Staining Kit
29021... 29128	Wheat Germ Agglutinin (WGA) CF® Dye Conjugates
31062	Yeast Vitality Staining Kit
31063	Yeast Viability Staining Kit
31064	Yeast Live-or-Dye™ Fixable Live/Dead Staining Kit
29068	ViaVac™ Red/Green (FUN®1)
31000	EvaGreen® Dye, 20X in Water
31019	EvaGreen® Dye, 2000X in DMSO
31077	EvaGreen® Plus Dye, 20X in Water
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31041... 99801	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31006	AccuBlue® High Sensitivity dsDNA Quantitation Kit with DNA Standards
31007	AccuBlue® Broad Range dsDNA Quantitation Kit with DNA Standards
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit with DNA Standards
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, cell viability assays and stains, and nucleic acid quantitation kits.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.