Revised: January 4, 2023

Product Information

PMA (Propidium Monoazide)

Product List

Cat. No.	Format	Unit Size
40013	Solid	1 mg
40019	20 mM in Water	100 uL

Storage and Handling

PMA solid (Cat. No. 40013) should be stored at 4°C or -20°C protected from light. When stored as recommended, the solid dye is stable for at least one year from date of receipt. If you are using PMA solid (Cat. No. 40013), briefly centrifuge the vial to collect the contents from the cap before reconstitution. With the lights dimmed, add 98 uL sterile dH_2O to the 1 mg vial of PMA and mix to prepare a 20 mM stock solution.

PMA, 20 mM in Water (Cat. No. 40019) and reconstituted solutions of Cat. No. 40013 should be stored at -20°C protected from light. When stored as recommended the dye solution is stable for at least six months from date of receipt or reconstitution.

Molecular Information: $C_{27}H_{32}Cl_2N_6$
CAS number: 91416-20-5
Molecular Weight: 511.5 g/mol
Color and Form: Red solid for 40013 and red liquid for 40019
Solubility: Soluble in water

Spectral Properties

λ_{abs} = 464 nm (before photolysis)
λ_{abs} /λ_{em} = ~510/~610 nm (following photolysis and reaction with DNA/RNA)

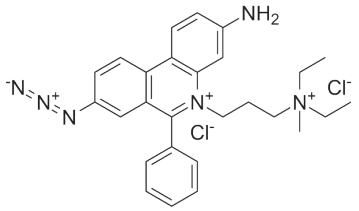

Figure 1. PMA (Propidium Monoazide).

Product Description

PMA (propidium monoazide) 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 PMA allows differentiation of live and dead cells using qPCR or other DNA amplification methods (Figure 4). v-PCR has been validated in a wide variety of bacterial strains, as well as yeasts, fungi, viruses, and parasites.

PMA binds to dsDNA with high affinity. Upon photolysis, the dye covalently reacts with DNA, resulting in permanent DNA modification. PMA 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. PMA 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 (Nocker *et al.* 2006). This feature makes the dye highly useful in the selective detection of viable cells by quantitative real-time PCR.

Biotium offers PMA Enhancer for use with Gram-Negative Bacteria (Cat. No. 31038) and now also offers a next-generation vPCR dye called PMAxx™ (Cat. No. 40069) with improved live/dead discrimination in bacteria compared to PMA. Visit www.biotium.com for more information.

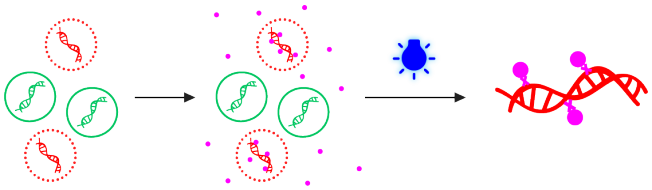

Figure 2. Principle of PMA modification of dead cell DNA. The cell membrane-impermeant PMA dye (purple dot) selectively enters dead cells with compromised membranes (red) and, after light treatment, covalently modifies the DNA. Subsequent PCR amplification of PMA-modified DNA templates is inhibited, allowing selective quantitation of DNA from viable cells (green).

Considerations for Viability PCR

- Viability 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 viability PCR. However, some methods, such as UV light exposure, may not immediately result in disrupted cell membranes (Nocker *et al.* 2007). Literature searches and pilot studies may help to determine whether viability PCR will work with your chosen cell type and killing method.
- It is advisable to choose the appropriate dye for your viability PCR experiment. Biotium offers three different viability 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.
- If your sample of interest is a gram-negative bacterium, you may want to use PMA Enhancer for Gram Negative Bacteria (Cat. No. 31038). This product is added to the sample during the viability PCR process, and improves the discrimination between live and dead cells. However, if your sample is a gram-positive strain, or a mixture of gram negative and gram positive, the Enhancer should not be used.
- Viability PCR requires a photoactivation step in order for the dye to covalently bind to the dead cell DNA. The PMA-Lite™ LED 2.0 Photolysis Device (Cat. No. E90006), designed for use with 1.5 mL tubes, is the most efficient device for performing this function. Other blue or white light sources with wavelengths spanning 465-475 nm 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. So care must be taken to avoid excessive heating of the sample.
- 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. We recommend using an amplicon of at least 100 bp. If detection of only one species or strain of bacteria is desired, you will want to design or find specific primers. We offer a variety of kits that include species-specific primers (see Related Products, page 4). It is advisable to validate your primers and PCR set up with genomic DNA from the same cell type before beginning your v-PCR experiment.
- If you wish to detect different bacterial strains simultaneously, primers against rRNA targets that are known to be pan-species-specific are a good choice. rRNA gene pyrosequencing has been used in combination with v-PCR for microbial population analysis, see PMA and PMAxx™ References for examples.

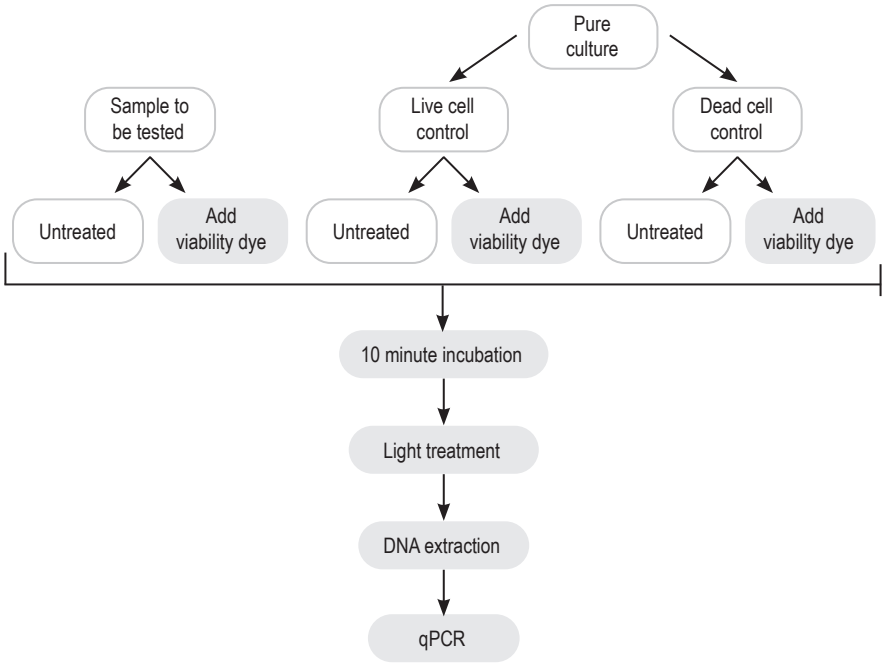

Figure 3. Viability PCR workflow overview, with recommended live and dead cell controls.

- Freezing of samples prior to performing viability 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 is recommended before freezing your samples prior to dye treatment. Samples can be frozen after dye treatment and photolysis.
- Part of the proposed mechanism of action of PMA is the removal of PMA-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.
- To validate PMA effectiveness in your sample of interest, it is best to perform live cell and dead cell controls, each with and without PMA (Figure 3). The change in Ct (dCt) caused by PMA for each control should be assessed (see "Data Analysis" on page 3).
- 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 PMA treatment.
- We recommend preparing stock solutions of PMA in water. In some publications, users have prepared their PMA stock solution in DMSO, however, this is not necessary. Unlike the older viability dye EMA, which is not soluble in water, PMA and PMAxx™ are very water soluble.

Before You Begin

- Read the "Considerations for Viability PCR" section on page 1 to determine the appropriate viability dye, primers, Enhancer, 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 PMA, pipetting PMA, and incubating with PMA should be done with the lights dimmed.

Standard Viability PCR Protocol

See Figure 3 for an overview of the procedure. This is a general protocol for treating cultured bacteria with PMA or PMAxx™. We recommended that you first perform a control experiment with live and dead controls for your specific organism.

1. Inoculate your desired media broth with bacteria and culture bacteria overnight, or longer, until the OD600 of the culture is approximately 1. Culture volume will depend on the size of the experiment; you will need 800 uL of culture for each condition to be tested (see step 3).
2. Suggested: To prepare dead cell control samples, heat inactivate bacteria at 90°C for 5 min. If it is desired to compare viability 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.
3. Pipette 400 uL aliquots of bacterial culture into clear microcentrifuge tubes. For each sample you will need one tube for PMA-treated cells and one tube for untreated (no dye added) cells in order to calculate dCt (see Figure 3, "Considerations for viability PCR," and "Data Analysis" for details).
4. If using PMA Enhancer for Gram-Negative Bacteria, add 100 uL of 5X Enhancer to each tube for a 1X final Enhancer concentration.
5. Working in low light, add the appropriate volume of PMA stock for a final concentration of 50 uM (e.g., 1 uL of 20 mM stock in 400 uL).
6. Incubate tubes in the dark for 10 minutes at room temperature. Perform incubation on a rocker with tubes covered with foil for optimal mixing.
7. Expose samples to light to cross-link PMA to DNA.

   Notes:
   a. For best results, we recommend that photo-crosslinking be carried out on Biotium's PMA-Lite™ 2.0 LED Photolysis Device (Cat. No. E90006). A 15 min exposure duration should be sufficient for complete activation.
   b. Commercial halogen lamps (>600 W) for home use have been employed for photoactivating PMA in some publications, though results may be less consistent due to inevitable variation in the set-up configurations. If you use a halogen lamp, we recommend that you lay tubes on a block of ice set 20 cm from the light source. The ice block should be in a clear tray with a piece of aluminum foil under the clear tray to reflect the light upward. Set the lamp so that the light source is pointing directly down onto the samples. Expose samples to light for 5-15 minutes.
8. Pellet cells by centrifugation at 5,000 x g for 10 minutes and remove the supernatant, taking care not to disturb the pellet.
9. Extract genomic DNA using your desired protocol or commercially available kit for your sample type.
10. Perform qPCR using primers against a chosen genomic DNA target for your organism of interest. Be sure to use the same volume of eluted DNA in each PCR reaction (i.e., do not normalize to ug of DNA). See "Considerations for viability PCR" for more information.

    Note: If you want to determine the absolute number of viable cells in your sample, you should also include genomic DNA of your cell type from a known cell number (See "Calculating the absolute number of viable cells" on page 3).

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.

Live & dead cell control dCt determination

1. After the qPCR run, use the instrument software to determine the threshold cycle (Ct) for each of your samples.
2. In order to determine whether PMA adequately inhibited amplification of dead cell DNA, calculate the delta Ct (dCt) for each of your control cell populations as shown:

   $dCt_{live} = Ct_{(live, PMA\text{-}treated)} - Ct_{(live, untreated)}$
   $dCt_{dead} = Ct_{(dead, PMA\text{-}treated)} - Ct_{(dead, untreated)}$
3. The expected result for the live cell control is a dCt close to 0 (+/- 1) (Figure 4). This indicates that PMA treatment did not affect viable cell DNA amplification. If a larger dCt is seen for the live cell control, see Troubleshooting, page 4.
4. 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, page 4.

   Note: The dead cell dCt 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; whether Enhancer was used. We have found that at 25 uM PMA, the dCt ranges from ~5 to 9, depending on the bacterial strain. In yeast, we have obtained dCts of 5, 7, and 10 for 50, 100, and 200 uM PMA, respectively.

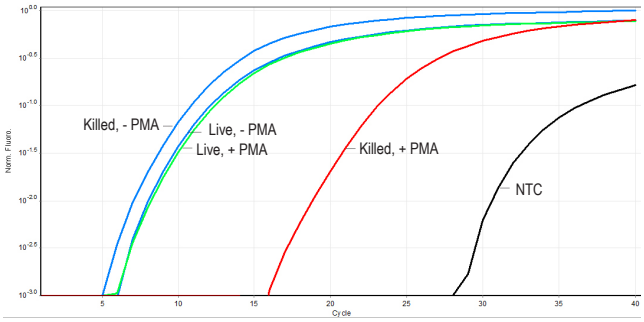

Figure 4. Normalized qPCR curves from a viability PCR experiment in which live and heat-inactivated *E. coli* were treated with PMA. qPCR was performed using primers against a region of the 16S rRNA gene. PMA treatment had no effect on amplification of DNA from live *E. coli*, but caused a dramatic delay in amplification of DNA from heat-killed *E. coli*.

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.

1. Calculate the dCt for the unknown samples as shown:

   $dCt_{sample} = Ct_{(sample, PMA\text{-}treated)} - Ct_{(sample, untreated)}$
2. You can convert the dCt into a percentage of viable cells as shown:

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

Calculating the absolute number of viable cells

If you want to calculate the absolute number of viable cells in your sample,* in the same experiment as your unknown samples, perform qPCR using genomic DNA from a titration series of known cell numbers for the cell type of interest to generate a standard curve of DNA vs. cell number. It is advisable that you have several gDNA dilutions that fall within the predetermined linear range of your qPCR assay.

1. 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. Calculate the cell number of your unknown sample as shown:

   Ct = slope(cell #) + y-intercept (y = mx + b)
   $Cell\ number_{sample}$ = (Ct - y-intercept) / slope

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

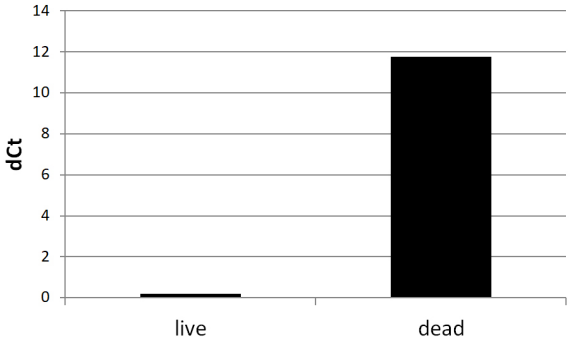

Figure 5. The dCt of live and killed *E. coli* with and without PMA treatment. The Ct threshold values from the qPCR curves in Figure 4 were obtained. The difference in Ct (dCt) between treated and untreated samples is a good way to evaluate the effectiveness of PMA in your samples. To calculate dCt, subtract the Ct of the untreated sample from the Ct of the PMA-treated sample ($Ct_{PMA\text{-}treated} - Ct_{untreated}$). Calculate dCt of your live cell control, dead cell control, and unknown samples.

PMA References

PMA and PMAxx™ from Biotium have been cited in hundreds of publications. View PMA and PMAxx™ References for an updated list of selected references.

First PMA publication: Nocker A., *et al.* Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. Microbiol. Meth. 67(2), 310-320 (2006).

PMA in bacteria with various killing methods: Nocker, A., *et al.* Molecular monitoring of disinfection efficacy using propidium monoazide in combination with quantitative PCR. J. Microbiol. Meth. 70, 252-260 (2007).

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 viability PCR experiment. Ensure that your chosen primers, master mix, and program work well. Increase the amount of template, if necessary.
High dCt seen in the live cell control sample.	The expected dCt value for a live cell control sample is less than 1. A higher-than-expected dCt value 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. 40051) to measure the membrane integrity of your cells. • Ensure that you are not using the PMA Enhancer with gram-positive bacteria. • Ensure that there is no detergent present in your samples. • Ensure that you are not freezing your samples prior to PMA 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.	The expected dCt value for a heat-killed dead cell control sample is greater than 4. A lower-than-expected dCt value in the killed cell control sample may be caused by several factors. • Confirm that your cells are actually dead. Use a dead cell stain such at Ethidium Homodimer III (Cat. No. 40051) to measure the membrane integrity of your cells. • Try a higher dye concentration: titrate the dye until you get an effective concentration that only alters dead cell DNA. • If your sample type is bacterial or viral, try using PMAxx™ (Cat. No. 40069) rather than PMA. • If your cells are gram-negative bacteria, try using the PMA Enhancer for Gram Negative Bacteria (Cat. No. 31038). • If you are using a light source other than our PMA-Lite™ LED Photolysis Device for photoactivation, we suggest PMAxx™ dye instead of PMA. Alternatively, 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 (i.e., do not normalize to ug of DNA (see “Considerations for viability PCR” on page 1 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 PMA concentration up to 100 or 200 uM.

Related Products

Cat. No.	Product
40069	PMAxx™ Dye, 20 mM in dH_2O
40015	Ethidium Monoazide Bromide (EMA)
E90006	PMA-Lite™ 2.0 LED Photolysis Device
31038	PMA Enhancer for Gram Negative Bacteria, 5X Solution
31033	PMA Real-Time PCR Bacterial Viability Kit – *Salmonella enterica* (invA)
31034	PMA Real-Time PCR Bacterial Viability Kit – *Mycobacterium tuberculosis* (groEL2)
31035	PMA Real-Time PCR Bacterial Viability Kit – *Staphylococcus aureus* (nuc)
31036	PMA Real-Time PCR Bacterial Viability Kit – *Staphylococcus aureus* (mecA)
31037	PMA Real-Time PCR Bacterial Viability Kit – *E. coli* O157:H7 (Z3276)
31041-31046	Forget-Me-Not™ qPCR Master Mixes
31050	PMA Real-Time PCR Bacterial Viability Kit – *E. coli* (uidA)
31051	PMA Real-Time PCR Bacterial Viability Kit – *Listeria monocytogenes* (hly)
31053	PMA Real-Time PCR Bacterial Viability Kit – *Legionella pneumophila* (mip)
31075, 31076	Viability PCR Starter Kits
40051	Ethidium Homodimer III, 1 mM in DMSO
40102	BactoView™ Live Green
40101	BactoView™ Live Red
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells
32000	Live Bacterial Gram Stain Kit

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent microbiology stains and viability assays, fluorescent CF® Dye antibody conjugates, and kits for cell biology research.

PMAxx, PMA-Lite, and their uses are covered by pending US and international patents.