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Produktinformation

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

PMA (Propidium Monoazide)

Formats

Catalog Number	Format	Size
40013	Solid	1 mg
40019	20 mM in Water	100 uL

Storage and Handling

PMA solid (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. To prepare a 20 mM stock solution, dissolve 1 mg PMA in 98 uL sterile dH_2O.

PMA, 20 mM in H_2O (40019) and reconstituted 40013 solutions 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.

Spectral Properties

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

Product Description

PMA (propidium monoazide) and the improved version PMAxx™ (40069) are high-affinity photoreactive DNA binding dyes 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 (Fig. 3). It has been validated in a wide variety of bacterial strains, as well as yeast, 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 now offers an improved alternative to PMA called PMAxx™ (40069). PMAxx™ provides the maximum amount of live/dead discrimination by v-PCR by further reducing the PCR signal from dead cell DNA. PMAxx™ works much better than PMA in all bacteria strains we have tested.

When using PMA for viability PCR of gram-negative bacteria, we recommend the use of PMA Enhancer for Gram-Negative Bacteria, 5X Solution (31038). When PMA Enhancer is added to gram-negative bacteria before treatment with PMA, dead cell DNA levels are further decreased, and thus live/dead cell discrimination is improved.

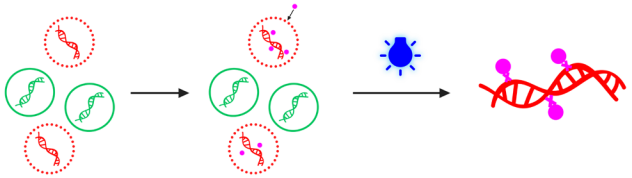

Figure 1. Principle of PMA modification of dead cell DNA. The cell membrane-impermeable PMA dye (purple dot) selectively enters dead cells with compromised membranes (red) and after light treatment, covalently modifies the DNA. Subsequent PCR amplification of PMA-modified DNA templates is inhibited,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

Considerations for viability PCR

1. 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.
2. It is advisable to choose the appropriate dye for your viability PCR experiment. Biotium offers three different viability PCR dyes: EMA, PMA, & 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 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 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 (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.
6. Viability PCR requires a photoactivation step in order for the dye to covalently bind to the dead cell DNA. The PMA-Lite™ LED Photolysis Device (E90002), designed for use with 1.5 mL tubes, is the most efficient device for performing this function. The Glo-Plate™ Blue (E90004) is a flat LED device that is best for photolysis of PMAxx™-treated samples. 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 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.
8. In order 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 (Fig. 2). The change in Ct (dCt) caused by PMA for each control should be assessed (see “Data Analysis”, p. 2).

Before You Begin

- Read the “Considerations for viability PCR” section above 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 in the dark.
- If you are using PMA solid (40013), spin down the vial prior to reconstitution. Working in the dark, add 98 uL of sterile water and mix. You may also resuspend the dye in DMSO, if desired.

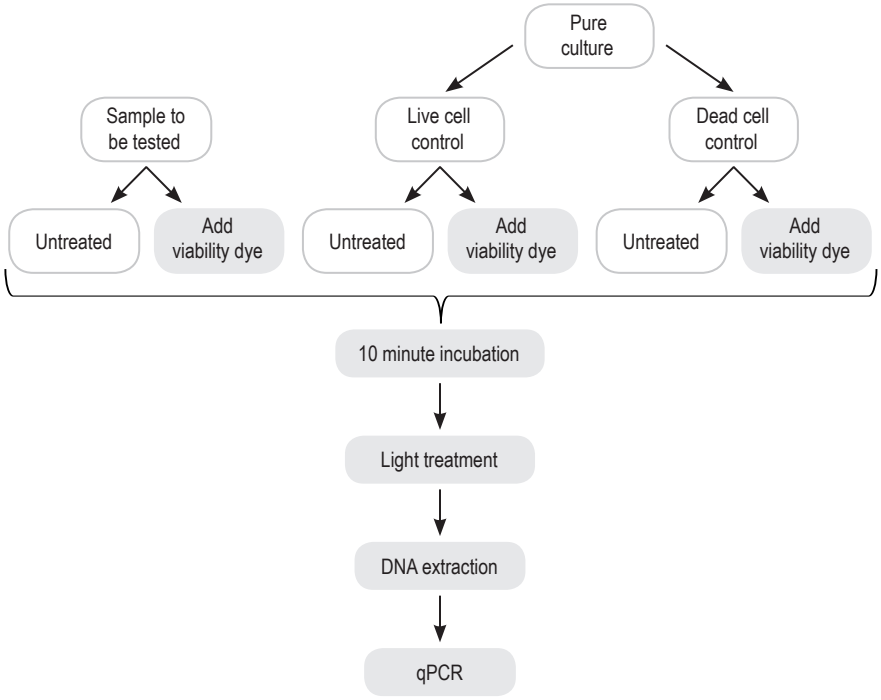

Figure 2. Viability PCR workflow overview, with recommended live and dead cell controls.

Standard viability PCR protocol

See Fig. 2 for an overview of the procedure. This is a general protocol for treating cultured strains of bacteria with PMA. 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 PMA treatment.

1. Inoculate your desired media broth with bacteria (volume is dependent on size of experiment). Culture bacteria overnight or longer until the OD_{600} of the culture is approximately 1.
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) in order to calculate dCt (see Fig. 2, "Considerations for viability PCR" #8, and "Data Analysis" for details).
4. (Optional, gram-negative bacteria only): Add 100 uL of PMA Enhancer for Gram Negative Bacteria, 5X (31038), to a 1X final concentration.
5. Working in the dark, 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. 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" on the previous page for information on light sources.
8. Pellet cells by centrifuging at 5,000 x g for 10 minutes.
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. See "Considerations for viability PCR, #3" regarding primer selection. 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, #7" for more information.
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", next page).

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 set-up with genomic DNA from the same cell type before beginning your viability PCR experiment.

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) (Fig. 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, next page.
4. The expected result for the dead cell control is a dCt > 4 (Fig. 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, next page.
5. 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; 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, & 10 for 50, 100, & 200 uM PMA, respectively.

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:

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

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.

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

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

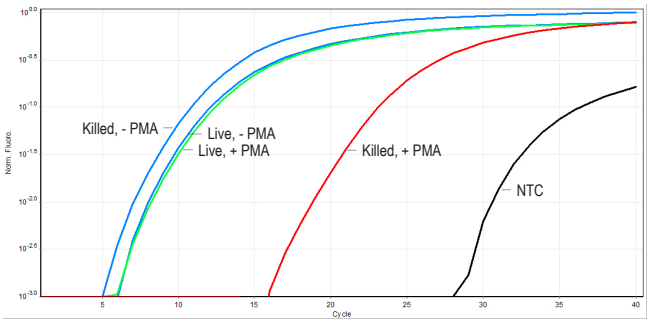

Figure 3. Normalized qPCR curves from a viability PCR experiment in which live and heat-inactivated *E. coli* were treated with PMA. qPCR was performed using primers against a region of the 16S rRNA gene. PMA treatment had no effect on amplification of DNA from live *E. coli*, but caused a dramatic delay in amplification of DNA from heat-killed *E. coli*.

PMA References

PMA from Biotium has been cited in hundreds of publications. For an updated list of selected references please check the PMA product page or the Viability PCR technology page on our website.

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).

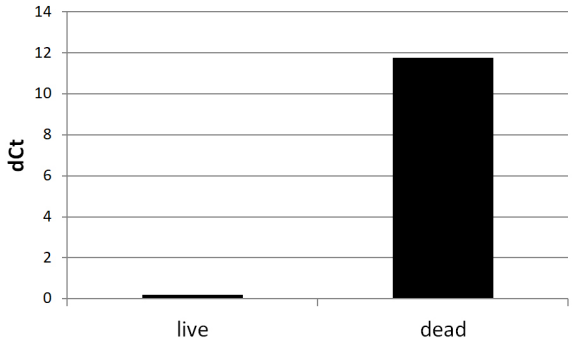

Figure 4. The dCt of live and killed *E. coli* with and without PMA treatment. The Ct threshold values from the qPCR curves in Figure 3 were obtained. The difference in Ct (dCt) between treated and untreated samples is a good way to evaluate the effectiveness of PMA in your samples. To calculate dCt, subtract the Ct of the untreated sample from the Ct of the PMA-treated sample ($Ct_{\text{PMA-treated}} - Ct_{\text{untreated}}$). Calculate dCt of your live cell control, dead cell control, and unknown samples.

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 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 (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.	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 (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™ (40069) rather than PMA. • If your cells are gram-negative bacteria, try using the PMA Enhancer for Gram Negative Bacteria (31038). • If you are using the Glo-Plate™ Blue (E90004), use 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 (ie, 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 PMA concentration up to 100 or 200 uM.

Related Products

Catalog number	Product
40069	PMAxx™ Dye, 20 mM in dH2O
40015	EMA (Ethidium Monoazide)
E90002	PMA-Lite™ LED Photolysis Device
E90004	Glo-Plate™ Blue LED Illuminator
31038	PMA Enhancer for Gram Negative Bacteria, 5X
31033	PMA-PCR Bacterial Viability Kit, Salmonella
31034	PMA-PCR Bacterial Viability Kit, M. tuberculosis
31035	PMA-PCR Bacterial Viability Kit, Staph. aureus
31036	PMA-PCR Bacterial Viability Kit, MRSA
31037	PMA-PCR Bacterial Viability Kit, E. coli O157:H7
31050	PMA-PCR Bacterial Viability Kit, E. coli
31051	PMA-PCR Bacterial Viability Kit, Listeria
31053	PMA-PCR Bacterial Viability Kit, Legionella
31075, 31076	Viability PCR Starter Kits
40051	Ethidium Homodimer III, 1 mM in DMSO (dead cell stain)
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.

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