BLU-V® Viability PMA Kit, Part 1

The BLU-V- Viability PMA Kit (cat. no. 296015) can be stored at 2–8°C until the expiration date.

Further information

- *BLU-V Viability PMA Kit Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- The BLU-V Viability PMA Kit is intended for molecular biology applications. The product is not intended for the diagnosis, prevention, or treatment of a disease
- Add 550 µl RNase-Free Water to each vial of PMA reagent, to obtain a 2.5 mM PMA solution. Mix by pipetting up and down 5 times or vortexing the tube for 4–6 sec, and centrifuge briefly. Keep the reconstituted PMA reagent protected from light until its use in protocol step 5.
- Prepare two 2 ml SafeSeal Micro Tubes with 1 ml of sample solution for each homogenous sample pool for the dead cell control samples. Close the tubes, label one tube as "Dead + PMA" and the other tube as "Dead No PMA". Heat for 10 min at 70°C in a thermomixer or shaking water bath to kill bacteria. Let tubes cool to room temperature. Continue with step 2. Treat the tube "Dead + PMA" according to the normal workflow, treat the tube "Dead No PMA" according to the descriptions for the "No PMA control sample". This dead cell controls will show the maximum capacity of PMA masking.

1. Add 1 ml of sample solution containing a concentrated mixture of live and dead target organism into a 2 ml SafeSeal Micro Tube.

   Note: For each experiment include at least one additional sample as a No PMA control sample. The "No PMA" control sample runs through the entire

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workflow but is not treated with the PMA reagent to serve as an unmasked C_T control in the subsequent real-time PCR reaction (see page 11 of *BLU-V Viability PMA Kit Handbook*). The C_T value of the No PMA control sample provides a point of comparison to determine the signal shift and to verify the correct application and performance of the PMA reagent.

2. Centrifuge the tube for 5 min at 13,000 x *g*.
3. Remove and discard the supernatant.

   Note: For fast and efficient disposal of the supernatant carefully decant the supernatant into a suitable waste container and softly tap the empty tube onto an absorbent tissue afterwards to remove any drops hanging at the rim of the tube.

4. Add 500 µl of Buffer EB to the pellet of the target organism, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.
5. Add 10 µl of the reconstituted PMA reagent to the samples. Do not add PMA reagent to the "No PMA" control sample (see step 1). Tightly cap the tubes, and mix by vortexing or inverting the tubes several times.
6. Place the tubes into the sample positions of the Incubation Box, close the lid and incubate the mixtures for 10 min at room temperature in the dark.
7. After incubation, transfer the sample tubes to the sample positions of the BLU-V System and illuminate the sample-PMA solution for 10 min. Vortex the sample tubes within the BLU-V System approximately every 2 min to allow a homogeneous sample illumination.
8. After light treatment, centrifuge at 13,000 x *g* for 5 min and discard the supernatant.
9. Continue BLU-V Viability PMA Kit, Part 2, step 10.