Preparation of reagents

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Abstract

For Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy protocol.

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Protocol

Stock stain solution

Step 1.
For SYBR stains, make a secondary stock by diluting the concentrated dye supplied by the manufacturer 10-fold with 0.02-µm filtered deionized water (dH_2O) and dispense into polypropylene screw-cap microcentrifuge tubes.

NOTES
Xu Zhong 02 Sep 2015
Stains should always be handled in low light to prevent photodegradation. Because the stains are sensitive to repeated freezing and thawing, the stains should be aliquoted as stock solutions in small volumes.

Stock stain solution

Step 2.
For Yo-Pro-1, dilute to 50 µM in an aqueous solution of 2 mM NaCN to prevent any microbial growth during the 48-h staining period.

NOTES
Xu Zhong 02 Sep 2015
Because the fluorescence of the dyes is very pH sensitive, it can be helpful to dilute the stain in TE buffer (pH 8) if processing strongly acid or basic samples (Chan, pers. comm.).

Stock stain solution

Step 3.
The diluted stains should be stored at –20°C, and ideally should be used within a week.

NOTES
Xu Zhong 02 Sep 2015
The dye should be checked before use to make sure that it has not precipitated or adsorbed to the walls of the storage tube. Adsorption of the stain to the tube walls is minimized when stored in polypropylene.

Stock stain solution

Step 4.
Each filter requires 2 µL SYBR stain; hence, 40 µL dispensed into each tube provides enough stain for

20 filters.

Stock stain solution

Step 5.

For Yo-Pro, freeze 800-µL aliquots for enough dye to stain 10 filters.

Glycerol/PBS solution

Step 6.

Prepare a solution of 50% glycerol and 50% PBS. The solution should be shaken or vortexed to ensure complete mixing, 0.02 µm filtered, and stored as a liquid at –20°C.

NOTES

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Alternatively, for long-term storage, 990 µL of the filtered mixture can be dispensed into microfuge tubes and frozen until ready for use.

Antifade (SYBR only)

Step 7.

Prepare a 10% stock solution of the antifade reagent by diluting 1 g phenylenediamine (PDA) in 10 mL of 0.02-µm filtered autoclaved dH_2O.

NOTES

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The PDA should go completely into solution, producing a colorless liquid If the stock solution is tea-colored or darker, it has oxidized and should not be used.

Antifade (SYBR only)

Step 8.

Dispense 500-µL aliquots of the working solution into microcentrifuge tubes and store at –20°C to minimize freezing and thawing.

NOTES

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The frozen reagent should be white; if it has a brownish tint it should not be used. It is possible to use other antifade reagents such as 0.5% (wt/vol) ascorbic acid in 50% (vol/vol) glycerol/PBS or SlowFade (Invitrogen), but they may provide less protection against fading (Noble and Fuhrman 1998). In contrast, DABCO (1,4-diazabicyclo[2.2.2]octane) in TE/glycerol is reported to be a superior antifade to PDA (Ortmann, pers. comm.).

Antifade (SYBR only)

Step 9.

Immediately before preparing the slides, make a 0.1% working solution of the antifade by adding the 10% phenylenediamine solution to the glycerol-PBS mixture.

Antifade (SYBR only)

Step 10.

Estimate 50 µL of reagent per slide.

Warnings

Reagents must be made in freshly prepared deionized 0.02-µm filtered water to prevent virus particles being introduced into the samples and causing high blanks.

Stains should always be handled in low light to prevent photodegradation. Because the stains are

sensitive to repeated freezing and thawing, the stains should be aliquoted as stock solutions in small volumes.