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Handling Reagents in the PCR Laboratory

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The exquisite sensitivity of PCR dictates that the reagents used in the PCR reaction must be free from all DNA contamination. Without this precaution, reliable results cannot be guaranteed. The fundamental rule that all PCR practitioners must adhere to is that ALL pre-PCR reagents must be prepared and maintained in an area free from all possible sources of DNA or RNA. Pre-PCR reagents can be divided into two classes: (1) reagents for sample preparation, and (2) reagents for amplification. Sample preparation reagents include buffers for the lysis of cells, extraction reagents for the release of nucleic acid, and resuspension buffers for the extracted nucleic acid. Post-PCR (detection) reagents are sequestered and handled in a different area of the laboratory. The purpose of this article is to provide a list of suggestions for the preparation and handling of PCR reagents.

PREPARATION OF PRE-PCR REAGENTS

1. All of these solutions should be prepared free from contaminating nucleic acids and/or nucleases (both DNases and RNases). To ensure that the nucleic acid preparations will routinely amplify properly, always use the highest quality components for each solution. This should help prevent problems due to the introduction of heavy metal ion contaminants, nucleases, or other unspecified contaminants. Gloves should be worn at all times when preparing reagents, handling samples, setting up reactions, and performing the subsequent detection of the amplified product(s).
2. The water used in all PCR reagents should be of the highest quality, freshly distilled/deionized, filtered, and autoclaved. Routine analysis of the water from the source tap should be performed to determine the conductivity of the water, as well as the possible contamination of the water supply by bacteria. Never assume that the supply is "clean." Significant bacterial contamination has been detected even in in-house distilled or deionized water systems that employ UV sterilization. It is important to remember that bacteria and algae grow in water storage systems (i.e., plastic water jugs); therefore, to minimize the chance of contamination of the water used for reagent preparation, always use freshly collected and processed water.
3. The addition of antimicrobials such as sodium azide is recommended for any reagents that will be stored from 20°C to 25°C. The presence of 0.025% sodium azide in amplification reagents or sample preparation reagents does not inhibit the amplification reaction.
4. All reagents should be made up in large volumes. Test to determine if the reagent performs satisfactorily, then aliquot into single-usage volumes for storage. Using aliquots of a proven reagent (stored under the appropriate condition) provides the user with consistency from experiment to experiment. The extraction of nucleic acid template should be performed with buffers or reagents that have not been previously exposed to other samples.
5. Always pipette sample preparation reagents with aerosol barrier (plugged) tips or positive displacement pipettes. Large volumes should be pipetted with individually wrapped, sterile disposable pipettes. The pipettors and pipette bulbs used in the reagent preparation area should be dedicated for this task and not used for any other purpose. A different set of dedicated pipettors and bulbs should be dedicated and sequestered for sample preparation use only.
6. Disposable, sterile bottles or tubes should be used for all reagent preparations.
7. New reagents should always be tested before new specimens are extracted with them. If possible, try to keep aliquots of a few samples that either can be used to demonstrate standard performance with a given set of reagents

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or, if the system is prone to difficulties, keep a representative problematic specimen to check reagent performance.

ESTABLISHMENT OF PCR CONTROLS AND REACTION PREPARATION

1. Ready-to-use "master mix" solutions can be prepared, aliquoted, and stored at either −20°C or 4°C. These master mix solutions have all but one of the necessary components for amplification to occur (i.e., no Mg^{2+} or no enzyme). Either a 10× or a 2× final concentration can be prepared and stored for easy, consistent use. This allows for experiment-to-experiment consistency, removing a significant chance for the introduction of experimental error by miscalculation or pipetting error when preparing complex reaction mixtures. Again, aliquoting of these reagents to single-use or two-time use only greatly enhances the chance of successful PCR by removing the possibility for inadvertent contamination of the reaction mixture. The pipettors for the preparation and dispensing of master mix should be dedicated for this use only and should be of the positive displacement kind.
2. As a rule, one should have a panel of negative, weak, and strong positive samples to use for checking the cleanliness and efficacy of the sample preparation reagents. In addition, the final resuspension buffer should be tested with known low copy numbers (20–50 copies) of the target sequence to demonstrate that there is no inhibition. If one is preparing a large batch of reagents for sample preparation, the reagents should be "clean tested" by amplifying at least 10–20 no-DNA controls with and without carryover control (i.e., ±UNG) to distinguish between the possibility of target versus amplicon contamination.
3. The reagents needed for the PCR amplification reaction also need to be handled in a manner that will prevent contamination with extraneous nucleic acids or nucleases. Components should be stored according to manufacturers' instructions in an area that is sequestered away from either sample preparation or detection activities.
4. Each laboratory or department must make a decision as to whether or not the synthesis of the primers and probes for their assays will be performed internally or prepared externally. If the synthesis capabilities exist internally, both synthesis and the subsequent purification of the primers must be located in an area removed from post-PCR activities, sample preparation, and plasmid DNA preparation. Any of the above-mentioned activities can lead to the inadvertent contamination of the primers with DNA that will be impossible to remove and potentially cause spurious results. Again, the pipettors used for handling of primers should be dedicated to pre-PCR reagent handling only and aerosol barrier tips should be used to prevent cross-contamination.

POST-PCR

Post-PCR reagents are used for the detection of amplified products. Routine detection methods include ethidum bromide staining of either acrylamide or agarose gels, radioactive probe detection following Southern transfers or dot blots, or nonisotopic detection methods (colorimetric microwell, reverse dot blots, chemiluminescent hybridization, protection assays). These techniques will be described in a subsequent supplement.

1. It is imperative that all reagents, disposables, and equipment used in post-PCR activities be solely dedicated to this purpose. Never use any equipment or reagents from this area in any pre-PCR activity.
2. The traffic flow in any lab that performs nucleic acid amplification reactions should be unidirectional, always moving from clean to dirty (i.e., pre-PCR to post-PCR).