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Technical Note

MagSi-NA Pathogens MSP and PurePrep 96 Nucleic Acid Purification System

Recommended use of internal controls

Introduction

MagSi-NA Pathogens MSP is intended for automated isolation of nucleic acids (DNA and RNA) from repiratory samples. The sample preparation procedure includes magnetic sample pooling of up to 6 samples without diluting the isolated nucleic acids. The obtained nucleic acids can be used directly as template for downstream applications such as PCR, qPCR, RT-qPCR or any kind of enzymatic reaction.

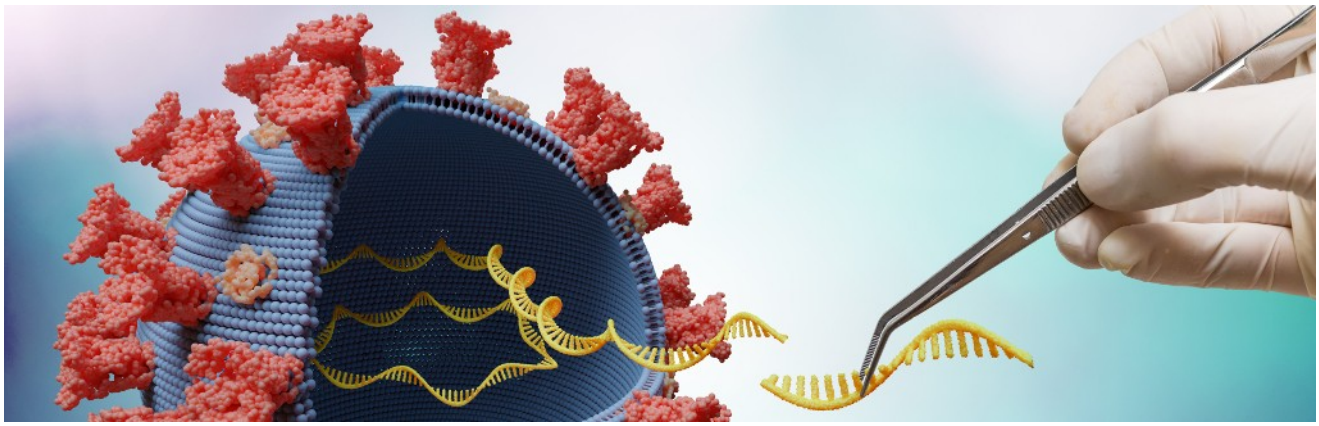
The PurePrep 96 instrument is a magnetic particle processor which uses magnetic rods that collect and transfer magnetic particles across microplates with a turntable-based design, eliminating the need for multiple pipette tips. Carefully designed rod covers prevent cross-contamination and allow for reproducible and efficient sample mixing and magnetic particle resuspension.

Most PCR assay kits include an internal control that is used to monitor every individual sample during the extraction process and detect potential PCR inhibitors. Purified samples from MagSi-NA Pathogens MSP include nucleic acids from up to 6 samples. During the pooling process, internal controls are also collected into a single sample. Most PCR assays have a normal variation of 0.5-1 Ct values, and are not accurate enough to detect a 2-fold difference in concentration of the internal control. As a result, a failure of purifying internal control from up to 3 out of 6 pooled samples could go undetected. It is therefore recommended to apply a different strategy for internal controls.

The current technical note describes two alternative procedures for using internal controls.

Internal control for monitoring PCR inhibition

The most simple procedure involves only monitoring PCR inhibition. In this case, the internal control should be added to each sample in the last sample plate within the magnetic sample pooling procedure. Depending on the pooling size, this could be sample plate 6, 5 or 3. If the internal control fails to produce a Ct value within the acceptance criteria, all sample results pooled into the well in the elution plate belonging to that PCR reaction must be considered invalid. If however, a mistake is made by the user leading to loss of lysis/binding efficiency for a sample included in sample plates 1-5 (e.g. pipetting error), the extracted and pooled sample will still produce a Ct value for the internal control and may lead to a false negative result for the targeted genetic sequence.



Using internal control to detect systematic extraction failures

Systematic mistakes in nucleic acid extraction procedures may involve accidentally omitting or exchanging kit components. For instance, if the user accidentally does not add Lysis Working Solution to a specific sample plate, nucleic acid from these samples may not be released and bound to magnetic beads. This would lead to ineffective extraction from the specific sample plate, while nucleic acids from sample plates before and after in the magnetic sample pooling procedure will still be extracted successfully.

Alternatively, the user may forget to add components to a specific sample plate that are required to achieve binding conditions. This can lead to ineffective binding of nucleic acids from this sample plate, and also to loss of previously bound nucleic acids.

In both cases, the difference in Ct value of the internal control will go largely unnoticed. Moreover, if Ct acceptance of internal controls is done in an automated fashion, it will lead to false negative PCR results for the targeted genetic sequence.

Systematic mistakes in nucleic acid extraction procedures described above can be detected by omitting the internal control in 5 out 6 samples. When doing so, the user must ensure that each sample plate includes a small set of samples that are spiked with the internal control, while other samples are not. Furthermore, the spiked samples cannot be pooled with other samples including internal control. In this way, each pooled sample should produce a Ct value which is equal to the internal control concentration after a nucleic acid extraction procedure without magnetic sample pooling. In the illustration to the right, an example is given for using the internal control in this way. A similar procedure can be used for 5:1 and 3:1 pooling. For more information, please contact support@magtivio.com.

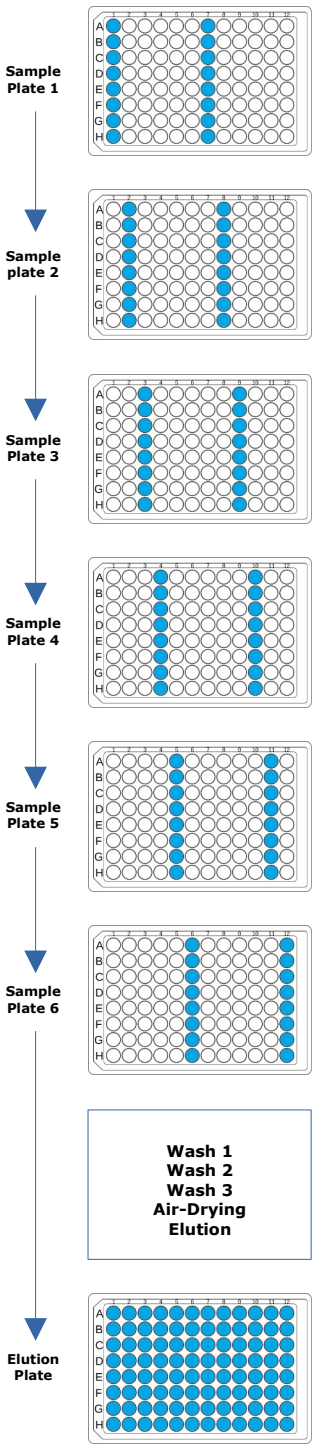


Fig.1: Using internal control in magnetic sample pooling. Blue wells indicate samples with internal controls.

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