ViralSEQ™ Mouse Minute Virus (MMV) Detection Assay

Integrated sample preparation and real-time PCR assay for the detection of Mouse Minute Virus (MMV) in cell culture samples

Features

- Detection of all known Mouse Minute Virus (MMV) strains
- Proven TaqMan® technology for highly sensitive detection down to 10 DNA copies per reaction (viral particles equivalent to 0.002 $TCID_{50}$)
- Specific, no cross-reactivity with unrelated DNA
- Discriminatory positive/extraction DNA control to eliminate possibility of false positive test results from accidental cross-contamination with control DNA
- Use with PrepSEQ™ 1-2-3 Nucleic Acid Extraction Kit, which is optimized for highly efficient DNA recovery
- Rapid time-to-results, typically in less than 4 hours
- Part of the Cell Culture Rapid Methods Program

The Threat of MMV Contamination

MMV contamination is a potential threat to CHO and NS0 cell culture manufacturing processes. Multiple cell culture manufacturing facilities have been negatively impacted over the past 20 years following contamination with this virus. Use of a rapid, real-time PCR–based detection method enables in-process monitoring throughout the cell culture manufacturing process and helps to enable the earliest possible detection of a contamination event.

The Applied Biosystems® ViralSEQ™ MMV Detection Assay is an integrated DNA purification and real-time PCR–based test for rapid, sensitive detection of MMV from a wide range of sample types. First, viral DNA is isolated from the sample using PrepSEQ™ magnetic beads. Highly sensitive and specific detection of the viral DNA is then performed using TaqMan® real-time PCR technology. The assay also includes a proprietary positive control molecule that eliminates the possibility of a false positive test result arising from inadvertent contamination of the test sample with positive control.

Detection of All Known MMV

MMV is a member of the Parvovirus family and is a nonenveloped virus approximately 20 nm in diameter. MMV contains a single-stranded DNA that is roughly 5 kb in size. There are four strains of MMV (i, p, m, and c) and seven genomic sequences reported in the NCBI database. The ViralSEQ™ MMV Detection Assay was designed to help enable detection of all known strains and genomes.

Rapid Time-to-Results Facilitates Routine In-Process Testing

The ViralSEQ™ MMV Detection Assay has an easy workflow that can deliver results in just under 4 hours (Figure 1). The assay can be used to perform routine in-process testing, enabling multiple MMV tests throughout the cell expansion process. The rapid assay time allows the earliest possible detection of MMV contamination and helps enable rapid assessment of the contamination scope (such as testing bioreactors, related batches or lots, raw materials, and the facility). The scope contamination event can be bracketed immediately, and the affected equipment areas and/or suspect raw materials can be quarantined. In addition, rapid real-time PCR testing can be used to confirm decontamination and to provide verification of viral DNA removal prior to restarting the cell culture processes. The versatile protocol also accommodates testing of nontypical samples, including surface swabs, sampling ports, and probes. The ViralSEQ™ MMV Detection Assay streamlines the surveillance process for the earliest possible detection of recontamination (Figure 2).

Figure 1. Workflow for the ViralSEQ™ MMV Detection System.

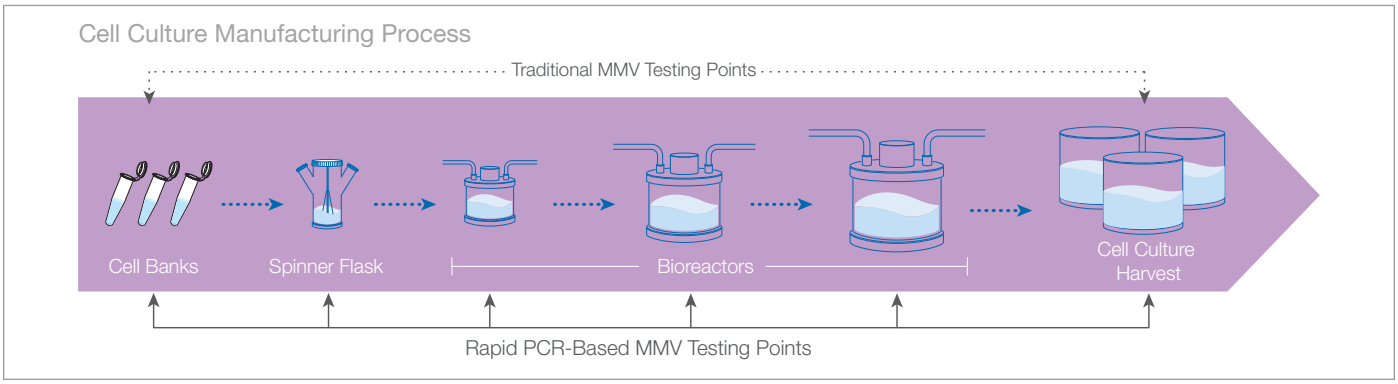

Figure 2. Sampling Points for MMV Detection. Rapid PCR-based testing for MMV infection can be conducted throughout the cell culture manufacturing process, from inoculation through harvest.

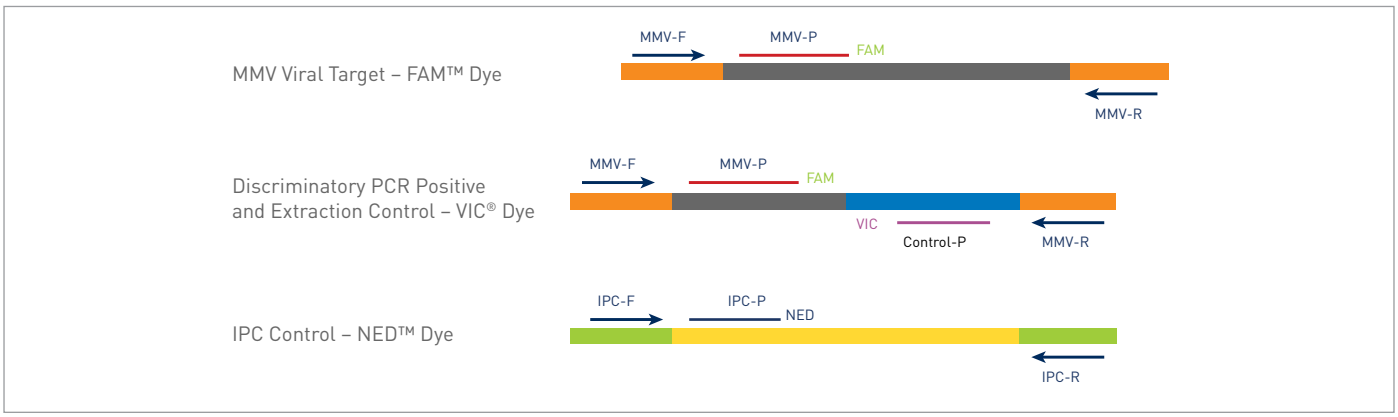

Figure 3. Multiplex Assay and Control Design. The ViralSEQ™ MMV Detection Assay design includes detection of target, internal positive control, and discriminatory control.

Discriminatory Positive Control

The ViralSEQ™ MMV Detection Assay also includes the MMV Discriminatory Positive/Extraction Control, a plasmid DNA containing the MMV DNA sequence that is recognized by the MMV detection assay (FAM™ probe). This control (Figure 3) was designed to behave like MMV DNA in both the sample preparation and detection portions of the assay. It has been engineered, however, to incorporate an additional probe (VIC® probe) binding sequence. The presence of both FAM™ and VIC® signal in a PCR reaction can be used to discriminate a positive test result between MMV (FAM™) and the control DNA (FAM™ and VIC®). This novel control enables risk-free DNA spike control testing, eliminating the possibility of a false positive test result due to accidental cross-contamination of a test sample with the positive control DNA.

Proven Sensitivity and Specificity

The assay is highly sensitive, able to detect as few as 10 copies of MMV genomic DNA per PCR reaction, which is equivalent to 0.002 $TCID_{50}$. Analysis of 10-fold serial dilutions of MMV genomic DNA demonstrates the broad dynamic range and efficiency of the assay (Figures 4 and 5).

Table 1 shows the ViralSEQ™ MMV Assay to be specific to the MMV genomic DNA target with no cross-reactivity with unrelated DNA.

Highly Efficient Recovery of MMV Genomic DNA Using the PrepSEQ™ 1-2-3 Sample Preparation Kit

The PrepSEQ™ 1-2-3 Nucleic Acid Extraction Kit is designed to help enable consistent and high percentage recovery of the MMV genomic DNA sample from complex sample types that include CHO cell culture and bovine serum. Figure 6 shows the C_t values of DNA extracted from the spiked sample were comparable to unspiked control that was amplified in the real-time PCR reaction, demonstrating the highly efficient DNA recovery using the PrepSEQ™ 1-2-3 Kit.

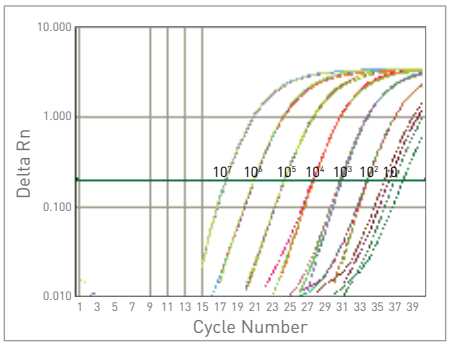

Figure 4. High Sensitivity and Broad Dynamic Range. The ViralSEQ™ MMV Assay can detect from 10^7 down to 10 copies of MMV genomic DNA per reaction.

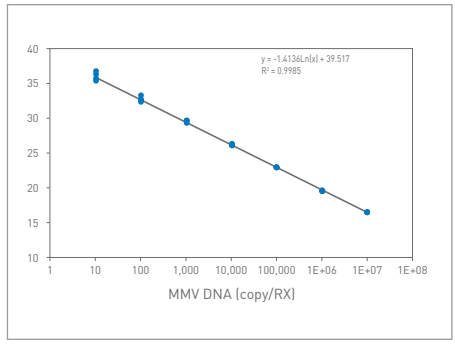

Figure 5. High PCR Efficiency. The ViralSEQ™ MMV Assay demonstrated high real-time PCR efficiency and a broad linear detection range.

Table 1. Common Unrelated DNA Exclusion Panel for the ViralSEQ™ MMV Detection Assay. The ViralSEQ ™ MMV Assay is able to discern between target DNA and unrelated DNA at likely levels experienced in a testing environment. Several sources of DNA commonly found in labs were tested.

DNA	Results
Human 10 ng	Undetected
Mouse 500 ng	Undetected
Rat 370 ng	Undetected
CHC 500 ng	Undetected
S. pneumoniae 10 ng	Undetected
S. cerevisiae 10 ng	Undetected
S. enterica 10 ng	Undetected
S. aureus 10 ng	Undetected
E. coli 10 ng	Undetected

Cell Culture Rapid Methods Program

The ViralSEQ™ MMV Detection Assay is part of the Cell Culture Rapid Methods Program, designed to streamline the detection of three common contaminants of mammalian cell culture–based biopharmaceutical manufacturing. The program sets new workflow standards in efficiency and product quality, combining one sample preparation step with real-time PCR–based assays for the detection of Mycoplasma, Vesivirus, and MMV on one instrument platform.

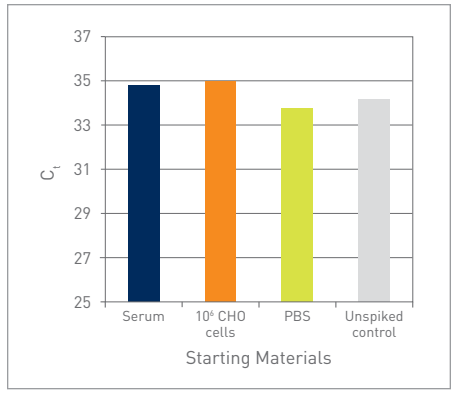

Figure 6. The PrepSEQ™ 1-2-3 Nucleic Acid Extraction Kit Delivers Reliable MMV Genomic Purification From Various Starting Materials. Two thousand copies of genomic DNA were added to serum, CHO cells, and PBS, extracted using the PrepSEQ™ 1-2-3 Kit, and detected using the ViralSEQ™ MMV Detection System. Regardless of starting material, the C_t values were consistent, showing the efficiency of the system.

ORDERING INFORMATION

Description	Size	Part Number
ViralSEQ™ Mouse Minute Virus (MMV) Detection Assay Contains real-time PCR assay, buffer and enzyme mix, positive control, and negative control	100 rxns	4444415
PrepSEQ™ 1-2-3 Nucleic Acid Extraction Kit Buffers, magnetic beads, and enzymes	100 rxns	4452222

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Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.327.3002
www.appliedbiosystems.com

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