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Your Data: Synthesizing Control RNAs for Real-Time RT-PCR Viral Quantification



MEGAscript® High Yield Transcription Kit TURBO DNase™ Enzyme MEGAclear™ Kit

Dr. Luke T Daum and colleagues (Longhorn Vaccines & Diagnostics, San Antonio, Texas) use real-time RT-PCR to quantify viral copies present in nose and lung homogenates from cotton rats (*S. hispidus*) challenged with influenza A virus. This article describes how they made the experimental controls using Ambion® kits and reagents.

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Relative Quantitation and Controls

Relative Quantitation (RQ; also called Comparative C_T) is an established method for using real-time RT-PCR to measure change in levels of a target nucleic acid sequence (e.g., influenza A) relative to the same sequence in a known calibrator sample. The exogenous control is a distinct nucleic acid sequence (e.g., influenza B) of known concentration added to each sample and serves as a reference to control for variations from extraction and pipetting. For Dr. Daum's experiments, calibrator and exogenous control complementary RNA (cRNA) were synthesized for normalizing viral concentration and controlling for sample variation, respectively.

Synthesis of Controls

To make the control cRNA, Dr. Daum and colleagues used RT-PCR to incorporate an overhanging 5' T7 promoter sequence into influenza A and B amplicons. The MEGAscript® T7 Transcription Kit was then used to generate RNA

transcripts from these amplicons. The resulting RNA was treated with TURBO DNase™ enzyme to destroy template DNA, and purified using the MEGAclear™ Kit to remove salts, buffers, primers, and enzymes.

The calibrator and exogenous control RNA were stored in THE RNA Storage Solution and used as reliable and consistent calibrator and exogenous control template RNA for quantifying influenza A virus from cotton rat nose and lung homogenates (Figure 1). Real-time RT-PCR was performed using RQ with the Applied Biosystems 7500 Real-Time PCR System.

ORDERING INFORMATION	CAT#	SIZE
MEGAscript® High Yield Transcription Kits		
T7 Kit	AM1334	40 rxns
T3 Kit	AM1338	40 rxns
SP6 Kit	AM1330	40 rxns
TURBO DNase™ Enzyme (2 U/μL)	AM2238	1000 units
MEGAclear™ Kit	AM1908	20 rxns
THE RNA Storage Solution	AM7000	10 x 1 mL
Applied Biosystems 7500 Real-Time PCR System	Various	1 instrument

Visit www.appliedbiosystems.com for more information.

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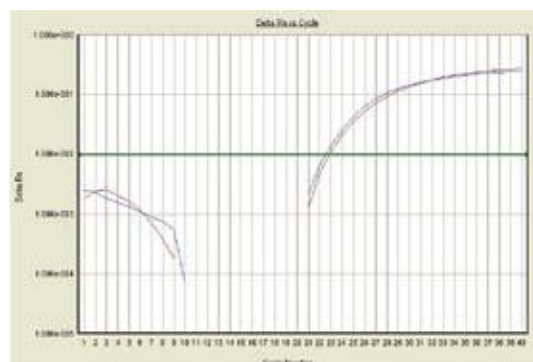


Figure 1. Real-Time RT-PCR Assays Detect Known Amounts of Calibrator and Exogenous Control Template. The calibrator and exogenous control reactions represent 0.1 pg of template cRNA corresponding to approximately 5.8×10^5 influenza copies. The calibrator and exogenous control were used in subsequent real-time RT-PCR to quantify viral copy number from cotton rat nose and lung samples infected with influenza A virus.

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