DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30329

MRT-PCR Purification using Exonuclease

Influenza Division, Virology, Surveillance and Diagnosis Branch, Genomics and Diagnostic Team

1.0 Purpose

1.1 The purpose of this procedure is to describe clean-up of amplified MRT-PCR product.

2.0 Definitions

2.1 EXO I: Exonuclease I

3.0 Critical Equipment

3.1 Multichannel Pipettes

3.2 Centrifuge

3.3 Thermocycler

3.4 4°C Refrigerator

3.5 -20°C Freezer

4.0 Laboratory Materials

4.1 Aerosol barrier pipette tips

4.2 0.2 ml PCR reaction 8-tube strip and 96-well plate

4.3 Sterile, nuclease free 1.5 ml micro-centrifuge tubes

4.4 Sterile plate sealing foils

4.5 Exonuclease I (20U/µL)

4.6 Nuclease-Free water (not DEPC-treated)

5.0 Safety Precautions

5.1 Adhere to the safety guidelines provided in the Biosafety in Microbiological and Biomedical Laboratories and follow all established site-specific safety procedures, including wearing proper personal protective equipment (PPE).

6.0 Procedure

6.1 Make Exonuclease (Exo) I master mix by aliquoting the following master mix into a sterile 1.5 micro-centrifuge tube.

Exonuclease I (Exo I) Master Mix	Single reaction	Full 96-well plate
Exonuclease I	0.13 µL	17.2 µL
10X Buffer (supplied in Exo kit)	0.20 µL	27.5 µL
Water	1.67 µL	230.3 µL
Total Volume	2.00 µL	275 µL

6.2 For efficient pipetting, aliquot Exo I master mix evenly into 0.2 mL strip tubes.

6.3 Using a multichannel pipette and a new tip for each reaction aliquot 2 µL of Exo I master mix into each 25 µL MRT-PCR product.

6.4 Seal plate securely with foil and briefly spin reaction plate in centrifuge.

6.5 Place reaction plate on thermocycler under the “EXO” program with cycling parameters:

Temperature	Time	Repeat
37°C	15min	1x
80°C	15min	1x
10°C	∞	1x

6.6 After cycling, the reaction place can be stored in short-term (overnight to 1 week) at 4°C or long-term (over 1 week to 1 month), store it in -20°C.

6.7 Any remaining Exo I master mix can be stored at -20°C.

7.0 Related Procedures

7.1 LP-328 – Multi-segment Reverse Transcription-PCR (MRT-PCR) of Influenza A and B Viruses