

Virus Cryopreservation Using a CoolRack® Module

Introduction

Snap freezing, or flash freezing, is the process by which samples are lowered to temperatures below -70°C very rapidly using dry ice or liquid nitrogen. Snap freezing achieves the same endpoint as slow rate-controlled freezing, but at a much faster rate. (-10 to -1000°C per minute, as compared to -1°C/minute) Snap freezing with a CoolRack module will provide sample vessel stability, organization and consistent freezing parameters, rapid hands-free sample processing while avoiding lost or contaminated samples. Snap freezing is performed on a pre-cooled CoolRack module, which ensures fast heat transfer. This method can provide excellent specimen integrity and a wide array of options for analysis, including extraction of proteins, DNA and RNA for use in research and diagnostics.

This procedure is intended to ensure that virus samples collected will be frozen in a safe and efficient manner while eliminating the risks of contamination and variation in molecular integrity. This protocol describes a general procedure for long-term storage of viruses. For each specific virus specie or strain, always refer to laboratory SOP.

Materials

- Virus preparation
- Cryoprotective agent (special cases only)
- CoolBox™ CFT30 ice-free cooling system
- CoolRack® CFT30 module
- Cryolabels and/or cryomarkers
- ThermalTray™ LP platform (optional)
- 50 mL reagent reservoirs
- CoolSink® LX55 reagent reservoir holder (optional)
- TruCool® cryogenic vials
- TruCool® hinged cryostorage box
- 37°C waterbath
- -80°C freezer
- Dry Ice

Virus Preparation

Follow the laboratory protocol for viral growth and/or purification. Refer to Centers for Disease Control and Prevention (CDC) guidelines for utilization of pathogens in specific Biosafety Level (BSL). *Pathogens are infectious agents and should always be manipulated under a biosafety cabinet with laminar flow.*

Virus Freezing

Non-enveloped viruses, some DNA viruses and virus-like particles can be maintained stably at 4°C for a relatively long period of time. However, RNA and most enveloped viruses are extremely heat labile and need to be snap-frozen (frozen rapidly) and stored at -80°C for long-term storage. Please note that most viruses will suffer damage if storage temperatures exceed greater than -60°C.

1. As a general rule, maintain the viral preparation at 4°C by placing it in a reagent reservoir and place the reservoir on a thermo-conductive CoolSink LX55 module for uniform and stable cooling. Rest the CoolSink LX55 on ice directly, or on a ThermalTray LP platform for greater stability to minimize ice contact with the reservoir and its contents.
2. Dispense 1 mL of the virus preparation* (or desired amount) in a pre-labeled TruCool cryogenic vial. To avoid titer reduction, maintain the vials at 4°C in a CoolBox CFT30 ice-free cooling system. The CoolRack CFT30 cryogenic vial module inside the CoolBox standardizes vial temperature and reduces contamination and spill accidents by allowing one-handed opening and closing of the cryogenic vials while seated in the CoolRack CF-T30 module.

* Most virus preparations consist of virus supernatants from infected cells cleared of cell debris and therefore contain enough serum to

act as cryopreservants. However, some viruses need an extra cryopreservant agent such as a 50% sucrose. Please refer to your laboratory SOP or the literature to assess whether you need to add a special media or cryopreservant to the viral supernatant or preparation.

3. While virus samples are kept cold at 4°C in the CoolBox CFT30, place a second CoolRack CFT30 module on dry-ice and let it equilibrate to -78°C, which takes approximately five to ten minutes. **Note: with this protocol, there is no need to make a dry-ice/ethanol slurry.** Place the cryogenic vials directly in the wells of the pre-equilibrated CoolRack CFT30 module to snap freeze the samples. This will take approximately three to five minutes.
4. Transfer the frozen samples to a TruCool hinged cryostorage box and place it in the -80°C freezer for long-term storage.

Virus Thawing

1. Transfer the cryogenic vials from the -80°C freezer into a pre-equilibrated CoolBox CFT30 containing a green freezing cartridge inside it. This will keep the vial frozen and allow transport of the vials in a safe manner.
2. Place the vials directly in a 37°C water bath, and slowly manually agitate the vials to enable the thawing process. Right before the whole liquid is completely thawed remove the vial from the 37°C water bath and place it on a CoolRack CFT30 module which has been previously equilibrated on ice. Samples are now ready for desired experimental procedures or titer assessment (TCID₅₀, plaque assay, etc.).

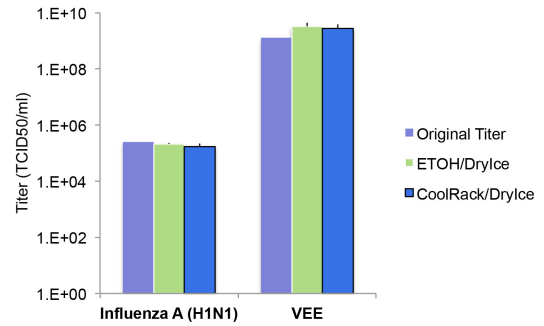


Figure 1: Graph showing the Titer (TCID₅₀/ml) of 2 different virus Influenza A and VEE using the 2 freezing methodologies compared to the original titer value