Protocol for Thawing Cryopreserved Cells

(December 2011)

I. Introduction

TZM-bl and 293T/17 cell lines are integral reagents for many aspects of the neutralizing antibody assay. Thawing the cryopreserved cells properly is crucial to ensure the viability and functionality of the cells throughout usage in the assays. When thawing cells it is important to remember to wear appropriate personal protective equipment (PPE) to minimize the risk of injury.

II. Definitions

GM: Growth Media

DMSO: Dimethyl Sulfoxide

CO_2: Carbon Dioxide

III. Reagents and Materials

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality than the recommended ones can be used whenever necessary.

293T/17 Cells
Vendor: American Tissue Culture Collection (ATCC)

TZM-bl Cells
Vendor: NIH AIDS Research and Reference Reagent Program

Growth Medium (see Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells**)**

Microliter pipettor tips, sterile
Vendor: Generic

Disposable pipettes, sterile, individually wrapped
Vendor: Falcon/VWR
1 ml pipettes
5 ml pipettes
10 ml pipettes
25 ml pipettes
50 ml pipettes

Cryogenic vials, 1.5 ml sterile screw cap
Vendor: Sarstedt Brand Products

Culture flasks with vented caps, sterile
Vendor: Costar/VWR

T-25 flask
T-75 flask

Face shield
Vendor: Generic

IV. Instrumentation

Recommended manufacturers are listed. Unless otherwise specified, equipment of equal or better quality than the recommended ones can be used whenever necessary.

Waterbath
Manufacturer: Precision Scientific

Biological Safety Cabinet
Manufacturer: Baker Co.

CO_2 Incubator
Manufacturer: Forma Scientific

Liquid Nitrogen Freezer / Dewar
Manufacturer: MVE

Light Microscope
Manufacturer: Olympus

Pipettor
Manufacturer: Rainin

V. Protocol

1. Thawing cells

NOTE 1: Be sure to wear a full-face shield during the handling of frozen specimens.

1.1 Transfer cryovials containing frozen cells from liquid nitrogen to a room temperature water bath in the biological safety cabinet.

1.2 If liquid nitrogen has seeped into the cryovial, loosen the cap slightly to allow the nitrogen to escape during thawing.

1.3 Hold the cryovial on the surface of the water bath with an occasional gentle "flick" during thawing. Do not leave the cryovial unattended during the thawing process. (It is important for cell viability that the cells are thawed and processed quickly – thawing only takes a few seconds).

1.4 Dry off the outside of the cryovials and wipe with a 70% ethanol solution before opening the vial to prevent contamination.

1.5 Transfer the contents of one vial of cells to a T-75 culture flask containing 30 ml of GM.

NOTE 2: It is important to dilute a cryoprotectant DMSO present in the cryovial at least 30-fold at this point to avoid cell toxicity.

1.6 Incubate the cells at 37°C/5% CO_2 overnight keeping the flasks in a horizontal position.

1.7 The next day, remove the medium and replace with 15 ml of fresh GM. Change the medium every 2-3 days until the cell monolayers are confluent.