

Protocol for Thawing Cryopreserved TZM-bl and 293T/17 Cells

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I. Introduction

TZM-bl, A3R5, M7-Luc 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

CO₂: 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

A3R5 Cells

Vendor: NIH AIDS Reagent Program

M7-Luc Cells

Vendor: Dr. Nathaniel R. Landau, The Salk Institute

Growth Medium (see Protocols: Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells; Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in A3R5 Cells; and Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in M7-Luc 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.

CO₂ 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. Also, frozen specimens should be placed in a closed container during transport from the freezer to the laboratory.

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% CO₂ overnight keeping the flasks in a horizontal position for adherent cells (293T/17 and TZM-bl) or a vertical position for suspension cells (A3R5 and M7-Luc).

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 cells are confluent.

VI. References

- I. Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells
- II. Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in A3R5 Cells
- III. Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in M7-Luc Cells