Protocol for Trypsin-EDTA Treatment for Disruption of Cell Monolayers

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I. Introduction

Adherent cell lines must be disrupted from cell culture flasks in order for them to be added to the neutralization assay or used for transfection for the production of pseudoviruses. It is critical that this procedure be done carefully because the cells can easily be damaged if left exposed to the Trypsin-EDTA for an extended period of time.

II. Definitions

GM: Growth Medium

DMEM: Dulbecco's Modified Eagle Medium

DPBS: Dulbecco's Phosphate Buffered Saline

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.

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

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

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

Trypsin-EDTA (0.25% trypsin, 1 mM EDTA), sterile
Vendor: Invitrogen

DPBS
Vendor: Invitrogen
Sterile

Disposable pipettes, sterile, individually wrapped
Vendor: Fisher
1 ml pipettes
2 ml pipettes
5 ml pipettes
10 ml pipettes
25 ml pipettes
50 ml pipettes

Culture flasks with vented caps, sterile
Vendor: Fisher
T-25 flask
T-75 flask

Trypan Blue (0.4%)
Vendor: Sigma

IV. Instrumentation

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

Biological Safety Cabinet
Manufacturer: NuAIRE

Incubator
Manufacturer: Forma Scientific

Pipettor
Manufacturer: Drummond Scientific Co.
PipetteAid XP

Light Microscope
Manufacturer: Olympus

Hemacytometer
Manufacturer: INCYTO

> ***NOTE 1:*** An automated cell counting devise (e.g., Countess, Manufacturer: Invitrogen) may be used in lieu of a light microscope / hemacytometer for cell counting and viability calculation.

V. Protocol

1. Trypsin-EDTA Treatment for Disruption of TZM-bl Cell Monolayers

> ***NOTE 2:*** TZM-bl is an adherent cell line that is maintained in T-75 culture flasks. Cell monolayers are disrupted and removed by treatment with Trypsin-EDTA at confluency when splitting cells for routine maintenance and when preparing cells for assay. Cells may be used for up to 60 passages in culture or 5 months, whichever comes first.

1.1 Remove the culture medium and eliminate residual serum by rinsing monolayers with 6 ml of sterile DPBS.

1.2 Slowly add 2.5 ml of a 0.25% Trypsin-EDTA solution to cover the cell monolayer.

1.3 Incubate at room temperature for 30-45 seconds.

1.4 Remove the Trypsin-EDTA solution and incubate at 37°C for 4 minutes. Do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.

1.5 Add 10 ml of GM and suspend the cells by gentle pipette action. Count cells (use the laboratory specific protocol on counting cells via hemacytometer or other cell counting device).

1.6 Seed new T-75 culture flasks with approximately 10^6 cells in 15 ml of GM. Cultures are incubated at 37°C in a 5% CO_2/95% air environment. Cells should be split upon confluency (approximately every 3 days).

1.7 Cells should be tested for Mycoplasma on a predetermined basis as defined in Protocol for Preparation of Cells for Detection of *Mycoplasma* Species.

2. Trypsin-EDTA Treatment for Disruption of 293T/17 Cell Monolayers

NOTE 3: 293T/17 is an adherent cell line that is maintained in T-75 culture flasks. Cell monolayers are disrupted and removed by treatment with Trypsin-EDTA at confluency.

2.1 Remove the culture medium and eliminate residual serum by gently rinsing the monolayers with 5 ml of sterile DPBS.

2.2 Slowly add 2.5 ml of a 0.25% Trypsin-EDTA solution to cover the cell monolayer. Incubate at room temperature for 30 seconds. Remove the Trypsin-EDTA solution and incubate at room temperature for 1 minute. Do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.

2.3 Add 10 ml of GM and suspend the cells by gentle pipette action. Count the cells with a hemacytometer or other cell counting device.

2.4 Seed new T-75 culture flasks with approximately 10^6 cells in 15 ml of GM. Cells should be split upon confluency (approximately 3 days).

2.5 Cell cultures should be tested for *Mycoplasma* contamination on a predetermined basis as defined in Protocol for Preparation of Cells for Detection of *Mycoplasma* Species.

3. Cell Viability with Trypan Blue

NOTE 4: When performing the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells (see Protocol for Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells) and Preparation and Titration of Env-pseudotyped viruses (see Protocol for Preparation and Titration of HIV-1 Env-pseudotyped Viruses), both TZM-bl and 293T/17 cells must be checked for cell viability with Trypan Blue or an equivalent method.

3.1 Dilute the cell suspension 1:10 (or a dilution that yields approximately 500,000 cells/ml) in sterile DPBS if counting the cells manually.

3.2 Mix equal parts of the cell suspension (e.g., 100 µl) with an equal volume of 0.4% Trypan Blue staining solution.

3.4 Load a hemacytometer or other counting chamber and count viable cells (viable cells are clear, dead cells are blue).

3.5 At least 80% of the cells must be viable in order to use the cells for transfection or a neutralization assay.