Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in A3R5 Cells

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I. Introduction

The preparation and maintenance of key reagents used for the Neutralizing Antibody Assay for HIV-1 in A3R5 Cells is crucial for obtaining accurate and reproducible results. Reagents must be created and stored as per manufacturer's guidelines and must be used within pre-established expiration dates.

II. Definitions

GM: Growth Medium

RPMI: Roswell Park Memorial Institute

FBS: Fetal Bovine Serum

DEAE-Dextran: Diethylaminoethyl-Dextran

HEPES: N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid

DMSO: Dimethyl Sulfoxide

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.

Complete Growth Medium for A3R5 Assay

RPMI-1640, with 25 mM HEPES and L-glutamine
Vendor: Gibco BRL Life Technologies
Sterile, store at 4°C

Fetal Bovine Serum
Vendor: Hyclone
Heat-inactivated 56°C for 30 minutes, 500 ml bottle, sterile. Store at −20°C. Once thawed, store at 4°C.

Gentamicin
Vendor: Sigma
Sterile, store at 4°C

DEAE-Dextran, hydrochloride, average Mol. Wt. 500,000
Vendor: Sigma

ViviRen Live Cell Substrate
Vendor: Promega
Store at −20°C

DMSO
Vendor: Sigma
Sterile, store at room temperature

IV. Instrumentation

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

Pipettor
Manufacturer: Biohit
Manufacturer: Rainin

Scale
Manufacturer: Mettler

4°C Refrigerator
Manufacturer: Sci-Cool

−20°C Freezer
Manufacturer: Sci-Cool

Low Temperature Freezer (at least −70°C)
Manufacturer: Thermo Labsystems

V. Protocol

1. Complete Growth Medium for A3R5 Assay

1.1. Complete GM for use in the A3R5 Neutralization Assay consists of RPMI-1640 medium containing 50 µg/ml gentamicin, 25mM HEPES, and 10% heat-inactivated FBS.

1.2. To make 500 ml of Complete GM, combine and mix in a sterile bottle:

447.5 ml RPMI-1640
50.0 ml FBS
2.5 ml gentamicin (10mg/ml)

1.3. Store the Complete GM at 4°C for up to 2 months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first). Before use in the assay, warm medium to 20°-37°C.

2. DEAE-Dextran

2.1. To prepare a 7.5 mg/ml solution, dissolve 3.75 gm of DEAE-Dextran in 500 ml of sterile water.

2.2. Create 10 ml aliquots in 15 ml sterile conical polypropylene tubes.

2.3. Store aliquots at -80°C.

2.4. DEAE-Dextran from some manufacturers does not have a listed expiration date. Contact the manufacturer for the stability of each DEAE-Dextran lot.

3. ViviRen Live Cell Substrate

3.1. Add 1 ml of DMSO to a vial containing 37 mg of the ViviRen powder. The concentration of the ViviRen substrate will be 60 mM after this step.

3.2. Aliquot the substrate in increments of 10 µl (i.e. 10, 30, 50) because 10 µl is needed for one 96-well plate.

3.3. Store the ViviRen Substrate at -20°C for up to 2.5 months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first).

3.4. Thaw aliquots immediately prior to use in the assay at room temperature.