

Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells (October 2021)

1.0 Introduction

The preparation and maintenance of key reagents used for the Neutralizing Antibody Assay for HIV-1 in TZM-bl 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.

2.0 Definitions

COA: Certificate of Analysis

DEAE-Dextran: Diethylaminoethyl-Dextran

DMEM: Dulbecco's Modified Eagle Medium

FBS: Fetal Bovine Serum

GM: Growth Medium

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

SDS: Safety Data Sheet

3.0 Reagents and Materials

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

Growth Medium

DMEM, with L-glutamine, sodium pyruvate, glucose and pyridoxine,

Manufacturer: Gibco BRL Life Technologies

Sterile, store refrigerated at 4°C

Fetal bovine serum

Manufacturer: Hyclone / Tissue Culture Biologicals / Nucleus Biologicals

Heat-inactivated 56°C for 30 minutes, 500 ml bottle, sterile. Store at -20°C. Once thawed, store at 4°C.

Gentamicin solution, 10 mg/ml

Manufacturer: Sigma

Sterile, store at 4°C

HEPES

Manufacturer: Gibco BRL Life Technologies

Sterile, store at 4°C

DEAE-Dextran, hydrochloride, average Mol. Wt. 500,000

Manufacturer: Sigma

Britelite Plus Reporter Gene Assay System

Manufacturer: Perkin Elmer Life and Analytical Sciences

Bright-Glo™ Luciferase Assay System

Manufacturer: Promega

15 ml conical polypropylene tubes

Manufacturer: Generic

50 ml conical polypropylene tubes

Manufacturer: Generic

4.0 Instrumentation

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

Pipettor

Manufacturer: Drummond

Scale

Manufacturer: Mettler

4°C Refrigerator

Manufacturer: LabRepCo

-20°C Freezer

Manufacturer: Sci-Cool / LabRepCo

Ultra Low Temperature Freezer (at least -70°C)

Manufacturer: Thermo LabSystems

5.0 Protocol

5.1 Growth Medium

5.1.1. Complete GM consists of DMEM containing 10% heat-inactivated FBS, 50 µg gentamicin/ml, and 25 mM HEPES.

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

- 435 ml DMEM
- 50 ml FBS
- 2.5 ml of gentamicin
- 12.5 ml of HEPES

5.1.3 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). Reagents used to prepare Complete GM must be labeled with the date that the sterile bottle is opened.

5.1.4 FBS bottles must be labelled with the date removed from the -20°C freezer to thaw and expiration date. FBS expires 4 months after thaw when stored at +4°C.

5.1.5 Before use in the assay, warm medium to 20°-37°C.

5.2 Antibiotic-free Growth Medium

5.2.1 Antibiotic-free GM consists of DMEM containing 10% heat-inactivated FBS and 25µM HEPES.

5.2.2 Example: To make 500ml of antibiotic-free GM, combine and mix in a sterile bottle:

- 437.5 ml of DMEM
- 50 ml of FBS
- 12.5 ml of HEPES

5.2.3 Store the antibiotic-free GM at 4°C for up to two months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first).

5.2.4 Before use in the assay, warm medium to 20°-37°C.

5.3 DEAE-Dextran

5.3.1 To prepare a 5 mg/ml solution, dissolve 2.5 gm of DEAE-Dextran in 500 ml of sterile water.

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

5.3.3 Store aliquots between -20°C to -80°C. Once thawed, store at +4°C - +8°C until it is exhausted.

5.3.4 Once the tube is thawed, label the date it was thawed and the date it was opened.

5.3.5 DEAE–Dextran used in the Neutralizing Antibody Assay Laboratory does not have an expiration date. According to the COA, the manufacturer recommends retesting every three years for stability from the Quality Release Date.

NOTE 1: It is important to note that conical tubes with DEAE-Dextran solution should be put for freezing in open racks, not placed in styrofoam racks. The freezing process begins at the exposed part of the tube. Consequently the shielded bottom of the tube if put in styrofoam rack can crack rendering the contents of the vial unusable.

5.4 Bright-Glo™ Luciferase Assay System

NOTE 2: The Britelite Plus Reporter Gene Assay system from Perkin Elmer Life and Analytical Sciences is an acceptable substitute for Bright-Glo. Please follow manufacturer's guidelines for preparation and use.

NOTE 3: Lyophilized Bright-Glo™ Substrate contains dithiothreitol and is classified as hazardous. Personal Protective Equipment (PPE) including gloves, lab coats, and eye protection must be worn when working with this reagent.

5.4.1 Lyophilized Bright-Glo™ Substrate should be stored at -20°C and Bright-Glo™ Buffer should be stored below 25°C. Use the reconstituted reagent on the day it is prepared, or store at -80°C for up to 1 month. An updated SDS and COA must be retained in the laboratory.

5.4.2 To reconstitute, transfer the contents of one bottle of Bright-Glo™ Buffer to one bottle of Bright-Glo™ Substrate and mix by inversion until the substrate is thoroughly dissolved.

5.4.3 Distribute 42.5mL of solution into 50mL labeled conical polypropylene tubes and store at -80°C for 1 month (or until expiration date of the reagent, whichever comes first). The tubes should be stored on a labeled rack/container in the freezer.

5.4.4 Thaw in a room temperature water bath (a water bath which is less than 25°C). The substrate should be allowed to reach room temperature before use. Avoid bright light.

5.4.5 After thawing, gently invert tube to mix solution prior to use. Excess reagent may be stored at -80°C and used through a maximum of 7 freeze-thaw cycles. Mark the tube with the number of freeze thaw cycles and volume in mL before refreezing.

NOTE 4: Manufacturer reports 10% loss of luminescence per 5 hours at room temperature, 10% loss per 24 hours at 4°C, and <5% loss after one month at -70°C.

5.5 Britelite Plus Reporter Gene Assay System

NOTE 5: The lyophilized Britelite Plus substrate is not classified as hazardous.

NOTE 6: All reagents must be stored according to manufacturer's specifications and within manufacturer's specified expiration dates. An up to date SDS and COA must be retained in the laboratory.

5.5.1 Equilibrate the Britelite Plus Substrate Buffer bottles to room temperature (about 30 minutes).

5.5.2 Pour one bottle of the Britelite Plus Substrate Buffer Solution (515ml) into two amber bottles of lyophilized Britelite Plus Substrate Solution bottles.

5.5.3 Recap the amber bottles and slowly mix (to prevent bubbles) the contents of the bottles by inversion until the substrate is completely dissolved (about 1 minute).

5.5.4 Pour the contents of both of the amber bottles back into the Britelite Plus Substrate Buffer Solution bottle.

5.5.5 Distribute 42.5ml of solution into 50ml labeled conical polypropylene tubes and store at -20°C for 1 month or -80°C for 3 months (or until the expiration date of the reagent, whichever comes first). The tubes should be stored on a labeled rack/container in the freezer.

5.5.6 Thaw in a room temperature water bath. Avoid bright light.

5.5.7 After thawing, gently invert the tube slowly to mix the solution prior to use. Use within 60 minutes of thawing. Excess reagent may be stored at -20°C or -80°C and used a maximum of 10 freeze/thaw cycles. Mark the vial with the number of freeze thaw cycles and the volume (in ml) remaining in the tube before refreezing.

NOTE 7: Stability of reconstituted Britelite Plus is approximately >85% remaining signal after 8 hours at 20°C.