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1.0 Purpose

This procedure describes a general method to be used to infect mammalian cells with viral vectors for the purpose of evaluating transgene expression.

2.0 Scope

This SOP is to be used for the infection of mammalian cells with viral vectors. The assay is to be performed by trained, IBC approved personnel under BioSafety Level (BSL)-2 conditions.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics (PA), has the authority to define this procedure.

3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA personnel are responsible for the implementation of this procedure.

3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials

4.1 Requestor-specified cell line as indicated on **Form 23117-01** (Infection Specifications and Approvals) grown to the specified density for infection.

4.2 Requestor-specified virus sample as indicated on QC Test Request Form, a null virus control (i.e., comparable virus not expressing protein of interest), and a positive control virus, if available. Unless indicated, virus samples should be stored at ≤ -70°C.

4.3 Appropriate BDP approved growth medium and infection medium as stated on **Form 23117-01**.

4.4 Fetal bovine serum (FBS), BDP PN 10109, or BDP approved equivalent, if required.

4.5 L-Glutamine, BDP PN 30373, or BDP approved equivalent, if required.

4.6 1X PBS without Ca^{++} or Mg^{++}, BDP PN 30007, or BDP approved equivalent, if required.

4.7 Trypsin-EDTA, BDP PN 30396, or BDP approved equivalent, if required.

4.8 Trypan Blue Stain, BDP PN 30890, or BDP approved equivalent.

4.9 Disposable pipettes: 1 mL BDP PN 20101, 2 mL BDP PN 20103, 5 mL, BDP PN 20104, 10 mL, BDP PN 20100, 25 mL, BDP PN 20102, 50 mL, BDP PN 20105, or BDP approved equivalent.

4.10 Aspirating pipettes, 2 mL, BDP PN 21331, or 5 mL, BDP PN 21330, or BDP approved equivalent.

4.11 Tissue culture flasks: 162 cm^2, BPD PN 20074, 75 cm^2, BDP PN 20745, or BDP approved equivalent.

4.12 Tissue Culture Dishes: 100 mm, BDP PN 21317, 6-well plates, BDP PN 20736, or BDP approved equivalent.

4.13 Cell scrapers, BDP PN 20660, or BDP approved equivalent.

4.14 Disposable centrifuge tubes: 15 mL conical centrifuge tubes, BDP PN 20006, 50 mL conical centrifuge tubes, BDP PN 20140, or BDP approved equivalent.

4.15 Sterile media bottles, BDP approved equivalent.

4.16 Cryovials, BDP PN 20007, or BDP approved equivalent.

4.17 Appropriate size/style BDP approved aerosol barrier pipet tips.

4.18 Dispatch, BDP PN 10167, 70% Sterile Isopropyl Alcohol, BDP PN 30129, Sporicidin, BDP PN 30135, Clorox bleach, BDP PN 10579, Cavicide, BDP PN 10168, or BDP approved equivalent per ***SOP 22909 Cleaning and Disinfection of Laboratories and Equipment in PA/BD***.

5.0 Equipment

5.1 Laminar Flow Biosafety Cabinet (BSC), suitable for BSL-2 containment.

5.2 Incubator, humidified, 36ºC ± 3ºC and 5% ± 2% CO_2.

5.3 Inverted light microscope (Zeiss or equivalent).

5.4 Water bath: 37°C ± 2°C.

5.5 Thermometer.

5.6 Benchtop centrifuge (Refrigerated if necessary).

5.7 Freezer: ≤-70°C.

5.8 Refrigerator: 2-8°C.

5.9 Micropipettors.

5.10 Pipet-aid automatic pipettor or equivalent.

5.11 Hemacytometer, BDP PN 20739, or BDP approved equivalent.

5.12 Vacuum flasks for aspiration of medium and samples.

5.13 Tray or equivalent, for transport of dishes.

6.0 Procedure

NOTE: Handle all viruses according to ***SOP 17109 Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious or Potentially Infectious Materials*.**

6.1 Verify that **Form 23117-01** (Infection Specifications and Approvals) has been completed and that the necessary approvals have been obtained. Once specifications have been established and approved for a project with a designated set of procedures, **True and Exact** copies of the completed, approved form may be submitted with each additional request unless any changes are required. If changes are required, a new form must be completed and approved prior to initiation of the assay.

6.2 Initiate a culture of the cell line indicated on **Form 23117-01** using the appropriate growth medium according to ***SOP 22140 Mammalian Cell Culture – Initiation and Maintenance of Cell Cultures in BQC***. Depending on the number of cells needed for infection, expand the cultures as necessary in appropriate tissue culture vessels.

6.3 Record reagents and equipment on **Form 23117-02**. Based on the number of cells/cultures to be infected (test sample and controls), calculate the amount of reagents necessary to perform the assay.

6.4 Seed an adequate number of tissue culture vessels for the test sample(s) and control(s) with additional vessels to perform cell counts if required. Use the seeding density indicated on **Form 23117-01**. Code appropriate plasticware to match workbook and label trays with the QC number and date. Incubate the cultures in a humidified incubator at 36°C ± 3°C and 5% ± 2% CO_2 as specified on **Form 23117-01**. Record cell preparation on **Form 23117-03**.

6.5 After the specified incubation length, confirm that the cells fall within the specified range for confluence or density. If a density is specified, perform a cell count on at least two representative vessels according to ***SOP 13214 Using a Hemacytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells***. Average the densities from all vessels counted. If the cell confluence or density falls outside of the specified range, notify the Area Supervisor prior to proceeding. Record assessment of confluence or cell count on **Form 23117-04**.

6.6 Dilute the virus samples (test samples and controls) in the specified medium to achieve the appropriate concentration for infection as indicated on **Form 23117-01**. If a sample concentration (e.g., vp/mL, pfu/mL, etc.) is specified, prepare the necessary concentration. If a multiplicity of infection (MOI) is specified, calculate the amount of virus needed to infect the cells based on the average density obtained from counting at least two representative vessels. Record sample preparation on **Form 23117-04**.

6.7 Dose the cells with diluted virus or medium (negative control) as specified on **Form 23117-01**. Incubate the cultures in a humidified incubator at 36°C ± 3°C and 5% ± 2% CO_2 for the specified incubation length.

NOTE: Some samples may require dosing in a minimal volume for a specified amount of time followed by the addition of growth medium. Some samples may require dosing in a fixed volume.

6.8 Harvest the cultures and process as specified on **Form 23117-01**. The desired harvest material may be the supernatant, the cells, or other (e.g., clarified cell lysate, both supernatant and cells harvested separately, etc.). Begin by harvesting the negative control (mock infected) cultures first, followed by null virus infected cultures (if applicable), the test sample infected cultures, and finally the positive control virus infected cultures (if applicable).

6.9 Store processed harvest samples as indicated on **Form 23117-01**.

7.0 Documentation

Generate and maintain all documentation relevant to this SOP according to ***SOP 21409 Good Documentation Practices***. Specific experimental details must be recorded in the attached templates (see attachments).

8.0 References and Related Documents

SOP 13214	*Using a Hemacytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells*
SOP 21409	*Good Documentation Practices*
SOP 22140	*Mammalian Cell Culture – Initiation and Maintenance of Cell Cultures in BQC*
SOP 22909	*Cleaning and Disinfection of Laboratories and Equipment in PA/BD*
SOP 17109	*Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious or Potentially Infectious Materials*
Form 23117-01	*Infection Specifications and Approvals*
Form 23117-02	*Reagents and Equipment*
Form 23117-03	*Cell Preparation*
Form 23117-04	*Infection and Harvest Procedure*

9.0 Change Summary

[redacted]