Biopharmaceutical Development Program

Standard Operating Procedure

Title: Infection of Mammalian Cells with Viral Vectors

SOP Number: 23117 **Revision Number: 02**

Supersedes: Revision 01 **Effective Date: JUN 2 2011**

Originator/Date: [redacted]

Originator/Date: [redacted]

Approval/Date: [redacted]

Approval/Date: [redacted]

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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).

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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.

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4.18 Dispatch, BDP PN 10167, 70% Sterile Isopropyl Alcohol, BDP PN 30129, Sporicidin, BDP PN 30135, Clorox bleach, BDP PN 20295, 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 22923, *Procedures for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel*.**

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.

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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

8.1 **SOP 13214** *Using a Hemacytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells*

8.2 **SOP 21409** *Good Documentation Practices*

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8.3 **SOP 22140** *Mammalian Cell Culture – Initiation and Maintenance of Cell Cultures in BQC*

8.4 **SOP 22909** *Cleaning and Disinfection of Laboratories and Equipment in PA/BD*

8.5 **SOP 22923** *Procedures for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel*

9.0 Attachments

9.1 **Attachment 1** Form 23117-01, Infection Specifications and Approvals

9.2 **Attachment 2** Form 23117-02, Reagents and Equipment

9.3 **Attachment 3** Form 23117-03, Cell Preparation

9.4 **Attachment 4** Form 23117-04, Infection and Harvest Procedure

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Attachment 1

NCI-Frederick
Form No.: 23117-01
SOP No.: 23117
Revision 02:

BDP Project #: _____ Product Description: _____

Requested Assay and Relevant Procedures **(include SOP#'s)**: _____

_____

Cell Line: _____ **If critical, indicated BDP PN and BDP LN:** _____

Tissue Culture Vessel for Infection (e.g., 100 mm dish, 6-well plate, etc.)

Description	BDP PN	# Vessels to infect/sample	Comments

Growth Medium: _____ **Infection Medium*:** _____

Component	BDP PN	Final Concen.

Component	BDP PN	Final Concen.

* If Infection Medium = Growth medium, indicate growth medium in blank and N/A component table.

Seeding density (e.g., cells/cm^2, cells/vessel, etc.): _____

Recommended Cell Incubation Length: Seed cells _____ day(s) prior to infection

Cells at Time of Infection: ___-___% Confluence and/or _____-_____ Density (e.g., cells/cm^2, or cells/vessel, etc.)

Virus Concentration or **MOI** (circle one)**:** _____ **Duration of Infection:** _____ ± ___ hours

Additional comments pertaining to Infection: _____

_____

Harvest Type (circle one): **Supernatant** or **Cells** or **Other** **If Other:** _____

Composition of Sample Buffer (if required): _____

Sample Harvest Procedure:
(Provide detailed description of sample harvest procedure, including any cell scraping, centrifugation steps, wash steps, use of sample buffers, freeze/thaw cycles, resuspension volume of final sample, etc. Specify the number and volume of any aliquots, as well as sample storage conditions. Include reference to method development/optimization captured in laboratory notebooks or on previous QC test requests. Attach additional pages as needed.)

_____
_____
_____
_____
_____
_____
_____
_____

Completed by: _____ **Date**:_____

Approvals:	**Project Scientist**: _____
(Signature/Date)	**Process Analytics**: _____
	Quality Assurance: _____

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Attachment 2

NCI-Frederick
Form No.: 23117-02
SOP No.: 23117
Revision 02:

Reagents and Equipment

Cell line: _____ Lot No.: _____ Passage No.: _____

Reagent	BDP PN	BDP Release #	Expiration Date
PBS w/o Ca++ and Mg++			
Trypsin/EDTA			

Reagent	Batch #	Expiration Date
Growth Medium:		
Infection Medium:		
Negative Control (diluent):		

Item	BDP Release #	Item	BDP Release #	Item	BDP Release #
1 mL pipets		15 mL tubes			
2 mL pipets		50 mL tubes			
5 mL pipets		Cryovial			
10 mL pipets		Cell Scraper			
25 mL pipets		100 mm dish			
50 mL pipets		6-well plate			
2 mL aspir. Pipets					

Equipment	MEF#	Calibration due date	Equipment	S/N	Calibration due date
CO_2 Incubator			Pipettor: _____		
BSC			Pipettor: _____		
Refrigerator			Pipettor: _____		
≤ -70°C Freezer			Pipettor: _____		
Waterbath		N/A			
Thermometer					
Microscope		N/A			
Centrifuge					

Recorded by: _____ Date: _____

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Attachment 2

NCI-Frederick
Form No.: 23117-03
SOP No.: 23117
Revision 02:

CELL PREPARATION

Trypsinize and count cells following ***SOP 22140, Mammalian Cell Culture – Initiation and Maintenance of Cell Cultures in BQC***. Follow ***SOP 13214, Using a Hemacytomter to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells.*** Do an appropriate dilution to count 200-300 cells, if possible. Count 10 squares of the hemacytometer (5 per side).

Cell Line: _____

Tissue Culture Vessel: _____ **Growth Surface Area**: _____

 # Vessels/Sample: _____ **Total # Samples:** _____

Total # vessels to seed*: _____

Target seeding density: _____

Volume to seed/vessel: _____ **Cell seed concentration (cells/mL):** _____

Minimum Total Volume to seed all vessels: _____

* # of vessels/Sample X Total # Samples + at least 2 additional vessels for cell counting, if required

_____ (cell count 10 squares) x ___ (dilution factor) x 10^3 = _____ cells/mL
(Cell Density)

_____ mL x _____ (cells/mL) = _____ cells
(Vol. seeding solution) (Cell seed conc.) (total cells needed)

Total cells needed: _____ cells = _____mL
Cell density: cells/mL (vol. cell density needed)

_____ mL - _____ mL = _____ mL
Vol. cells needed. (vol. medium) (Vol. seeding solution)

Performed By:_____ Date:_____

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Attachment 2

NCI-Frederick
Form No.: 23117-04
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Infection and Harvest Procedure

%Confluence or Cell Density
Indicate method of culture assessment (Circle one): **%Confluence** or **Density** ___days post-seeding
Complete the corresponding section below.

%Confluence:

Specified Confluence Range: _____ - _____%, Observed Confluence: _____%

If observed confluence falls within the specified range, proceed with infection. If not, consult with the Area Supervisor before proceeding.

Cell Density:

Trypsinize and perform a cell count on two representative vessels according to ***SOP 13214, Using a Hemacytomter to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells.*** Do an appropriate dilution to count 200-300 cells in 10 squares of the hemacytometer (5 per side), if possible.

Total final volume of cell suspension prepared from each vessel: _____

Vessel 1: _____ (cell count 10 squares) x ___ (dilution factor) x 10^3 = _____ cells/mL or _____

Vessel 2: _____ (cell count 10 squares) x ___ (dilution factor) x 10^3 = _____ cells/mL or _____

(_____ + _____)/2 = _____
(Vessel #1 Density) (Vessel #2 Density) (Observed Average Density)

Specified Density Range: _____, Observed Average Density: _____

If observed average density falls within the specified range, proceed with infection. If not, consult with the Area Supervisor before proceeding.

Comments: _____

_____

_____

_____

_____

_____

Performed By:_____ Date:_____

Make Additional Copies as Necessary.

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Attachment 4 (Continued)

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Infection and Harvest Procedure

Sample Preparation- Calculation of Minimum Volume and Virus

Indicate method of infection dose determination (Circle one): **Virus concentration** or **MOI**
Complete the corresponding section below.

Virus Concentration:

Specified concentration to dose: _____ Specified volume to dose/vessel: _____

Vessels to dose: _____ Total Minimum Volume needed: _____
(Vol. to dose/vessel X # vessels to dose)

Total Minimum Virus needed: _____
(Total min. vol. needed X Specified con. to dose)

Based on the number of vessels to be infected, dilute the sample in an appropriate volume of the specified infection medium. Use the space provided on the subsequent page to indicate sample dilution calculations.

Performed by: _____ Date: _____

MOI:

Specified MOI: _____ Observed Average Density: _____ Total # cells/vessel: *_____

Volume to dose/vessel: _____ # Vessels to dose: _____

Total Minimum Volume needed: _____

* Observed Ave. density
X Surface area of vessel
= Total # cells/vessel

_____ X _____ = _____
(MOI) (Total # cells/vessel) (total amount of Virus/Vessel)

_____ / _____ = _____
(total amount of Virus/Vessel) (Volume to dose/vessel) (Concentration of virus/volume)

_____ X _____ = _____
(Concentration of virus/volume) (Total Minimum volume needed) (Total Minimum Virus needed)

Based on the number of vessels to be infected, dilute the sample in an appropriate volume of the specified infection medium. Use the space provided on the subsequent page to indicate sample dilution calculations.

Performed by: _____ Date: _____

Make Additional Copies as Necessary.

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Attachment 4 (Continued)

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Infection and Harvest Procedure

Sample Preparation-Sample Dilution Calculation
Use the space provided to indicate sample dilution calculations.

QC#: _____ Sample Name: _____ Stock Concentration: _____

QC#: _____ Sample Name: _____ Stock Concentration: _____

QC#: _____ Sample Name: _____ Stock Concentration: _____

Performed By:_____ Date:_____

Make Additional Copies as Necessary.

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Attachment 4 (Continued)

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Infection and Harvest Procedure

Sample Dosing

Circle the appropriate options where indicated:

1. Prior to dosing: **remove growth medium** or **do not remove growth medium.**
2. Dose negative control vessels with appropriate volume of diluent/vessel: _____
3. Dose null virus control (if available) with appropriate volume/vessel: _____
4. Dose test sample virus with appropriate volume/vessel: _____
5. Dose positive control virus (if available) with appropriate volume/vessel: _____
6. Incubate in a humidified incubator at 36°C ± 3°C and 5% ± 2% CO_2 for: _____ ± _____ hours

Performed By:_____ Date:_____

7. Re-feed dosed cultures by:

   removing inoculum and adding ___ mL medium or **adding ___ mL medium** or **do not re-feed.**

8. After refeed, incubate cultures for _____ ± _____ hours. Check here if not refed: ____

Performed By:_____ Date:_____

Sample	Dose Time	Refeed Time*	Calculated Harvest Date/Time
Negative Control			

*N/A if refeed not performed.

Recorded By:_____ Date:_____

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Infection and Harvest Procedure

Sample Harvest

Sample Name	Harvest Time/Date	Total Hours Infection Time
Negative Control		

Harvest Type (circle one): **Supernatant** or **cells** or **Other:** _____
Complete corresponding section below.

Supernatant Harvest Procedure:
Unless otherwise indicated on Form 23117-01, collect medium from cultures, beginning with negative controls, null virus control, test samples, etc. Clarify any debris by centrifugation. Aliquot and store supernatant samples as specified.

Comments: _____

Volume of supernatant harvested: _____ Centrifugation (rpm/time/temp): _____
Aliquot size: _____ Storage conditions: _____

Cell Harvest Procedure
Perform cell harvest as specified on Form 23117-01. **Comments:** _____

Sample Buffer (if used): _____	Total Volume of final cell suspension: _____
Freeze/thaw cycles (if performed): _____	Centrifugation (rpm/time/temp): _____
Aliquot size: _____	Storage Conditions: _____

Performed By:_____ Date:_____

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Attachment 4 (Continued)

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Infection and Harvest Procedure

Sample Harvest (Continued)

'Other' Harvest Procedure:

Begin by defining **Other** (e.g., clarified cell lysate, cells and supernatants harvested separately, etc.) = _____

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Total Volume of harvest: _____ Centrifugation (rpm/time/temp): _____
Freeze/Thaw Cycles: _____ Sample Buffer: _____
Aliquot size: _____ Storage conditions: _____

Performed By:_____ Date:_____

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