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1.0 Purpose

This procedure describes a general method to be used to transfect mammalian cells with plasmid DNA.

2.0 Scope

This SOP is to be used for expressing protein in mammalian cells by transfection of a cell line with plasmid DNA.

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

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

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

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

3.5 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Equipment and Materials

4.1 Equipment

4.1.1 Laminar Flow Biological safety cabinet (BSC).

4.1.2 Incubator 37ºC ± 1ºC and 5% ± 2% CO2, humidified.

4.1.3 Inverted Microscope (Zeiss or equivalent).

4.1.4 Bench-top centrifuge (Refrigerated, if required).

4.1.5 Waterbath 37°C ± 2°C, as required.

4.1.6 Thermometer, as required.

4.1.7 Freezer: ≤-70°C or ≤-20°C, as required.

4.1.8 Refrigerator: 2-8°C.

4.1.9 Micropipettors.

4.1.10 Pipet-aid automatic pipettor or equivalent.

4.1.11 Hemacytometer, BDP PN 20739 or BDP approved equivalent.

4.1.12 Vacuum flasks for aspiration of medium and samples.

4.1.13 Tray or equivalent for transport of plates and/or dishes.

4.2 Materials

NOTE: Unless otherwise specified, an appropriate BDP approved equivalent may be substituted for or used in addition to the materials listed below.

4.2.1 Requestor-specified cell line as indicated on Form 22196-01 (Transfection Specifications and Approvals) grown to the specified density for transfection.

4.2.2 Requestor-specified plasmid sample as indicated on QC Test Request Form, and null plasmid control (e.g., empty vector), and a positive control/reference standard plasmid, if available.

4.2.3 Appropriate BDP approved growth medium and transfection medium as stated on Form 22196-01. [i.e., OptiMem I Reduced Serum Medium (BDP PN 30529).].

4.2.4 Requestor-specified transfection reagent as indicated on Form 22196-01 [i.e., Lipofectamine 2000 (BDP PN 30693), Fugene 6 (BDP PN 30581).]

4.2.5 1M Tris, pH 7.4, BDP PN 10042, if required.

4.2.6 Polysorbate (Tween) 20, BDP PN 10318, if required.

4.2.7 Protease Inhibitor Cocktail, BDP PN 30585, if required.

4.2.8 Fetal bovine serum (FBS), BDP PN 10109, if required.

4.2.9 L-Glutamine BDP PN 30373, if required.

4.2.10 PBS without Ca++ or Mg++ BDP PN 30007, if required.

4.2.11 Trypsin-EDTA BDP PN 30396, if required.

4.2.12 Trypan Blue BDP PN 30890.

4.2.13 Disposable pipets: 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.

4.2.14 Aspirating pipets, 2 mL BDP PN 21331, or 5 mL BDP PN 21330.

4.2.15 Tissue culture flasks: 162 cm2 BPD PN 20074, 75 cm2 BDP PN 20745, or other BDP approved vessel.

4.2.16 Tissue Culture Dishes: 100 mm BDP PN 21317, 6-well plates BDP PN 20736, or other BDP approved vessel.

4.2.17 Cell Scrapers, BDP PN 20660.

4.2.18 Cryovials BDP PN 20007.

4.2.19 Disposable centrifuge tubes: 15 mL conical centrifuge tubes BDP PN 20006, 50 mL conical centrifuge tubes BDP PN 20140.

4.2.20 1.5 mL sterile Eppendorf tubes (BDP PN 20659).

4.2.21 Sterile media bottles, BDP approved.

4.2.22 Aerosol Barrier Pipet tips, 100 μL (BDP PN 21484), 200 μL (BDP PN 20673), and 1 mL (BDP PN 20769).

4.2.23 Dispatch, BDP PN 10167, 70% Sterile Isopropyl Alcohol, BDP PN 30129, Sporicidin, BDP PN 30135, Clorox bleach, BDP PN 10579, Cavicide, BDP PN 10168, per ***SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC***.

5.0 Procedure

NOTE: In Process and Development testing does not require **Form 22196-01** to be approved prior to testing. If desired, the requestor may fill out **Form 22196-01** to indicate specific transfection parameters. In this case, **Form 22196-01** does not require pre-approval. Specific methods and data generated from development assays of a particular project will be used as supporting data in completing **Form 22196-01** for final approval by the Project scientist, PA and BQA.

5.1 Verify that **Form 22196-01** (Transfection 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.

5.2 Initiate a culture of the cell line indicated on **Form 22196-01** 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.

NOTE: Antibiotics should not be used as they can interfere with transfection.

5.3 Record reagents and equipment on **Form 22196-02**. Based on the number of cells/cultures to be infected (test sample and controls), calculate the amount of reagents necessary to perform the assay.

5.4 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 22196-01**. Code appropriate plasticware to match workbook and

label trays with the QC number and date. Incubate the cultures in a humidified incubator at 37°C ± 1°C and 5% ± 2% CO2 as specified on **Form 22196-01**. Record cell preparation on **Form 22196-03**.

NOTE: Some procedures require that the cells be seeded at the time of the transfection. Follow the specific procedure indicated on **Form 22196-01**.

5.5 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 22196-04**.

5.6 Assemble the transfection mixes as indicated on **Form 22196-01**. Pipet the appropriate volume of the specified medium into sterile Eppendorf tubes for each reaction. Add the appropriate volume of the specified transfection reagents to the medium taking care not to touch the sidewalls of the tube(s). Mix the contents by gently tapping the tube and incubate at room temperature for the specified amount of time. Add the appropriate volume/quantity of DNA to the medium/transfection reagent solution and mix by gently tapping the tube(s). Incubate the transfection mixes at room temperature for the specified amount of time. Record the necessary data on **Form 22196-04**.

5.7 Prepare the cell culture plates for transfection as indicated on **Form 22196-01**.

5.8 Add the transfection mixtures to the cell cultures as indicated on **Form 22196-01**. Transfecting the cells in duplicate (2 wells/transfection mix) is recommended; the duplicates can be used for analysis, if needed. Incubate the cultures in a humidified incubator at 37°C ± 1°C and 5% ± 2% CO2 as specified on **Form 22196-01**. Record the data on **Form 22196-04**.

5.9 After the specified incubation period, harvest the cultures and store the samples as indicated on **Form 22196-01**. Document the harvest on **Form 22196-04**.

NOTE: Some samples may require the use of the following Sample Extraction Buffer: 50 mM Tris, pH 7.4, 1% Tween 20, protease inhibitor cocktail. This is prepared by the dilution of 500 µL of 1 M Tris, pH 7.4 into 9.4 mL of water. A protease inhibitor cocktail is then dissolved into this solution. Once the tablet is completely dissolved, 100 µL of Tween 20 is added. This mixture is prepared fresh on the day of harvest and is kept chilled on ice or at 2-8°C.

6.0 Documentation

6.1 Generate and maintain all documentation relevant to this SOP according to ***SOP 21409 - Good Documentation Practices***. Specific experimental details must be recorded in the appropriate forms listed in section 7.0.

7.0 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 *Use, Cleaning and Disinfection of Equipment and Laboratories in PA/BD*

Form 22196-01 *Transfection Specifications and Approvals*

Form 22196-02 *Reagents and Equipment*

Form 22196-03 *Cell Preparation*

Form 22196-04 *Transfection and Harvest Procedure*