SOP Title:	**Suspension Cell Based Stability Testing of Retroviral Vectors**
SOP Number:	**23009**
Revision:	**02**

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1. PURPOSE

This SOP describes the materials and methods needed to measure the stability of retroviral vectors for cell therapies.

2. SCOPE

This SOP applies to BDP personnel performing suspension cell-based stability testing of retroviral vectors for chimeric antigen receptor-T cells (CART) or other cell therapy products.

The stability assay is used to detect transduced cells that express the gene of interest by the appropriate method stated in the attachments below.

3. RESPONSIBILITIES

3.1 Director, Process Analytics/Quality Control (PA/QC)

- Defines this procedure.

3.2 Process Analytics/Quality Control (PA/QC)

- Trains laboratory personnel.
- Performs this procedure.
- Reviews the results of this procedure.

3.3 Quality Assurance

- Provides quality oversight.

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
22670	12 well Tissue Culture Plate, Non-Treated	☒ YES ☐ NO
20006	Disposable centrifuge tubes 15 mL	☒ YES ☐ NO
22669	Cell culture flask, untreated, T75	☒ YES ☐ NO
21472	Pipet Tips 20µL	☒ YES ☐ NO
21767	Pipet Tips 250µL	☒ YES ☐ NO
20769	Pipet Tips 1000µL	☒ YES ☐ NO
20104	Disposable pipettes 5 mL	☒ YES ☐ NO
20100	Disposable pipettes 10 mL	☒ YES ☐ NO
20102	Disposable pipettes 25 mL	☒ YES ☐ NO
30437	RPMI 1640 medium	☒ YES ☐ NO
30891	Fetal Bovine Serum (FBS)	☒ YES ☐ NO
31392	200mM L Glutamine	☒ YES ☐ NO
31390	100mM Sodium Pyruvate	☒ YES ☐ NO
22577	0.22 µm 500ml Sterile Filter Bottle	☒ YES ☐ NO
22643	0.22 µm Syringe Filter	☒ YES ☐ NO
30890	Trypan Blue	☒ YES ☐ NO

Part Number	Description	BDP Approved Substitution Permitted?
31425	Vectofusin-1	☒ YES ☐ NO
10189	Water	☒ YES ☐ NO
30007	1X PBS	☒ YES ☐ NO
N/A	Jurkat cell line, or other suspension cell line as appropriate for the retroviral vector	☒ YES ☐ NO

5. EQUIPMENT

- CO_2 Incubator, Forma Scientific, Inc., or equivalent
- Biological safety cabinet, Baker (Model B6-0001), or equivalent
- Hemocytometer
- Microscope
- Water Bath
- Centrifuge
- Microcentrifuge

6. CELLS AND CULTURE

Perform all steps aseptically in a certified BSC, **SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC.** For cell recovery, propagation, medium preparation, and documentation, follow **SOP 22140 -Mammalian Cell Culture-Initiation and Maintenance of Cell Culture in Process Analytics/Quality Control.** For cell count and cell viability, follow **SOP 13214 - Using a Hemocytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells.** The volume of medium, samples, or reagents should be within ± 5% of indicated volume as below unless specified.

7. PROCEDURE

7.1 Begin Cell Culture

7.1.1 Record reagents and equipment used on Form 23009-01.

7.1.2 Make Complete Media by adding components on **Form 23009-01,** with the expiration date based on the component with the shortest expiration date as per **SOP 22140.**

7.1.3 Thaw appropriate cells and immediately mix with 10mL pre-warmed Complete Media.

NOTE: All cell-based work must be done in a biological safety cabinet.

7.1.4 Centrifuge the cells at 400xg for 5 minutes.

7.1.5 Remove excess media and resuspend the cells in 15mL of Complete Media.

7.1.6 Add the 15mL of cell/media mixture to a T75 flask and incubate in a cell incubator at 37°C and 5%CO_2. Record all incubation times on **Form 23009-02.**

7.1.7 After 48 hours ± 4 hours centrifuge the cells for 5 minutes at 400xg and resuspend the cells in fresh complete media.

NOTE: Currently, Jurkat cell lot [redacted] cells are resuspended in 20 mL of fresh complete media, and Jurkat cell lot [redacted] cells are resuspended in 40 mL of fresh complete media. The specific cell lots currently used for different viruses are listed in Attachment 1.

7.1.8 Add the cell/media mixture to a T75 flask and incubate in a cell incubator at 37°C and 5%CO_2. Record all incubation times on **Form 23009-02.**

7.2 Transduction

7.2.1 Pre-warm the centrifuge to 33°C ± 3°C by running it at 3500 RPM until ready for spinoculation.

7.2.2 After 72 hours ± 4 hours, perform a cell count.

7.2.3 In a microcentrifuge tube add 20µL of Trypan Blue.

7.2.4 Remove 20µL of cells and add to the 20µL of Trypan Blue.

7.2.5 Add 15µL of Trypan Blue/Cell mixture to a hematocytometer and count as specified on Form 23009-02 as defined by **SOP 13214.**

NOTE: If there are not enough cells to perform the assay, continue growing the cells. Repeat step 7.2 after approximately 24-48 hours.

7.2.6 Dilute cells to $0.625x10^6$ cells/mL as per **Form 23009-02** and add 0.8mL of cells to 9 wells of a 12-well tissue culture plate for a final cell count of $0.5x10^6$ cells/well.

NOTE: If the cell concentration is below $0.625x10^6$ cells/mL but the total cell number is enough to complete the assay, centrifuge the cells at 400xg for 5 minutes and resuspend in the correct volume of complete media to reach $0.625x10^6$ cells/mL.

7.2.7 Place the 12-well plate in the incubator at 37°C and 5%CO_2 while making the viral dilutions.

7.2.8 If not yet aliquoted; dissolve Vectofusin-1 in ultrapure water to a concentration of 1000µg/mL. Aliquot 50µL per microcentrifuge tube and store at -80°C.

7.2.9 Thaw 2x50µL aliquots of Vectofusin-1 and combine with 900µL of pre-warmed RPMI to make 100µg/mL Vectofusin-1.

7.2.10 Quickly thaw the retroviral vector in the water bath and prepare the retroviral dilutions in microcentrifuge tubes according to the "Initial Dilutions" column in the table in Attachment 1. For example, for 400µL of a 1:2.5 dilution add 160µL of viral vector to 240µL of RPMI.
NOTE: All the virus dilutions listed in the table will be tested at time point T=0. The virus dilution that shows the highest transduction efficiency at T=0 will be tested at subsequent time points. For GPC2 and CD19 retroviruses, all the virus dilutions listed in the table (0; 1:2.5; 1:5) will be tested at each stability time point.

7.2.11 In new microcentrifuge tubes combine 100µL of 100µg/mL Vectofusin-1 and 100µL of the appropriate viral dilution and incubate for 10 minutes at room temperature. For the negative (0) control, add 100µL of RPMI instead of the viral vector.

7.2.12 Remove the 12-well plate from the incubator and add 200µL of the Vectofusin-1/viral dilution mixture to the appropriate wells.

7.2.13 Centrifuge the 12-well plate in a pre-warmed centrifuge at 33°C ± 3°C for 1.5 hours.

7.2.14 Remove the plate from the centrifuge and incubate in a cell incubator at 37°C and 5%CO_2 for 24 hours ± 4 hours. Record all incubation times on **Form 23009-02.**

7.3 Media Change

7.3.1 Remove the 12-well plate from the incubator and transfer each well to a new microcentrifuge tube.

7.3.2 Centrifuge each tube for 5 minutes at 400xg.

7.3.3 Aspirate the supernatant and resuspend the cells in 1mL of pre-warmed complete RPMI.

7.3.4 Transfer the cells to a new 12-well plate.

7.3.5 Incubate the plate in a cell incubator at 37°C and 5% CO_2 for 48 hours ± 4 hours. Record all incubation times on **Form 23009-02.**

8. DATA ANALYSIS

8.1 Remove the 12-well plate from the incubator and transfer the cells from each well to new microcentrifuge tubes.

8.2 Setup a MACSQuant flow cytometer following the assay/SOP listed in Attachment 1 and proceed to determining transduction efficiency of cells by running the appropriate assay. The gating strategy will be modified to select the appropriate target.

8.3 Report transduction efficiency results on **Form 23009-03.** The virus dilution used to assess stability will be established during release testing. The virus dilution that shows the highest transduction efficiency will be established at time point T=0. At subsequent time points, the same virus dilution that was used at T=0 will be tested**.** The mean values for transduction efficiency will be reported**.**

NOTE: The highest transduction efficiency value of the tested dilutions is currently reported for GPC2 and CD19 Retroviruses. For all other retroviruses, the mean value for transduction efficiency will be reported.

9. DOCUMENTATION AND RECORDS

9.1 Record all reagents and equipment on appropriate assay forms.

9.2 Use **Form 23009-01** Suspension Cell Based Retroviral Stability Reagents and Equipment.

9.3 Use **Form 23009-02** Suspension Cell Based Retroviral Stability Assay Procedure.

9.4 Use **Form 23009-03** Suspension Cell Based Retroviral Stability Assay Results Summary.

10. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
12211	Instruction and Maintenance practices for the Nikon Eclipse E400 Microscope
13214	Using a Hemocytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells
22140	Mammalian Cell Culture – Initiation and Maintenance of Cell Cultures in Process Analytics/Quality Control
22909	Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC
22988	Determination of GPC2CAR Transduction Efficiency with the GPC2 Flow Cytometry Assay
23007	Determination of CD19 CAR Transduction Efficiency with the Anti-CD19-Idiotype Flow Cytometry Assay
22992	Determination of CD123XCD3 Transduction Efficiency with the human CD20 Flow Cytometry Assay
23008	Operation and Use of Olympus CKX53 Culture Microscope
23009-01	Suspension Cell Based Retroviral Stability Assay Reagents and Equipment
23009-02	Suspension Cell Based Retroviral Stability Assay Procedure
23009-03	Suspension Cell Based Retroviral Stability Assay Results
23014	Determination of CD22 TCR Transduction Efficiency with the mouse TCR beta Flow Cytometry Assay
23131	Operation of the MACSQuant Analyzer 10 Flow Cytometer

11. ATTACHMENTS

Attachment 1 Assay Analysis and Specification

Attachment 1 Assay Analysis and Specification

Retrovirus	Initial Dilution	Final Dilution	Cell Line Use	Transduction Assay	SOP	Specification
GPC2	0; 1:2.5; 1:5	0; 1:25; 1:50	Jurkat, Lot [redacted]	GPC2	22988	≥ 10%
CD19	0; 1:2.5; 1:5	0; 1:25; 1:50	Jurkat, Lot [redacted]	CD19	23007	≥ 15%
CD22 TCR	0; 1:2.5; 1:5	0; 1:25; 1:50	Jurkat, Lot [redacted]	mTCRb	23014	Positive for expression of the CD22 TCR
CD123XCD3 BiTE	0; 1:2.5; 1:5	0; 1:25; 1:50	Jurkat, Lot [redacted]	CD20	22992	Positive for expression of CD20