



BIOPHARMACEUTICAL DEVELOPMENT PROGRAM

SOP Title: Determination of Lentivirus Transduction Efficiency with the Protein L Flow Cytometry Assay
SOP Number: 22207
Revision: 02

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1. PURPOSE

This SOP describes the materials and methods needed to measure transduction efficiency for recombinant lentiviruses that direct the expression of chimeric antigen receptors (CAR). The assay uses the detection of CARs by Protein L interaction in a flow cytometry format

2. SCOPE

This SOP applies to BDP personnel performing the Protein L flow cytometry assay. The ability to detect lentivirus-transduced cells that express chimeric antigen receptors by Protein L depends upon the ability of the Protein L to access and bind to the kappa light chain (sub-types VkI, VkIII, and VkIV) in the ScFv region of the chimeric antigen receptor. If that interaction is sterically- hindered or otherwise compromised, the Protein L assay may not be used to determine transduction efficiency. The utility of the Protein L flow cytometry assay must be determined empirically for each new CAR construct or sequence modification.

3. RESPONSIBILITIES

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

- Defines the procedure.



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3.2 PA/QC Personnel

- Trains lab personnel.
- Performs procedure.
- Reviews data.

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
20006	15 mL Centrifuge Tube	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
20140	50 mL Centrifuge Tube	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
30652	Albumin, Human, 10% Solution	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
30656	Hanks' Balanced Salt Solution (HBSS)	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31168	FACS Tubes with Cell Strainer	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
31169	Recombinant Protein L	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31170	Anti-Protein L-FITC	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31171	MACSQuant Calibration Beads	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31172	MACSQuant Running Buffer	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31176	MACS Comp Bead Kit, anti-REA	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31199	Anti-CD3-PE-Vio 770, human, clone REA613	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31208	Anti-CD14-FITC, mouse, clone REA934	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
31212	Anti-CD33-PE, human, clone REA775	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO

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Part Number	Description	BDP Approved Substitution Permitted?
31213	7-AAD Staining Solution	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
31215	Anti-CD45-VioGreen, human, clone REA747	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO

5. EQUIPMENT

- MACSQuant Analyzer 10 Flow Cytometer, Miltenyi Biotec
- Biosafety Cabinet
- Refrigerator
- Eppendorf Centrifuge 5417C, or equivalent

6. PROCEDURE

- 6.1 Record reagents and equipment used on **Form 22207-01**.
- 6.2 Record the completion of each step on **Form 22207-02**.
- 6.3 Turn on the MACSQuant Analyzer 10 and allow the optical bench to warm up for at least 30 minutes, as described in **SOP 23131 Operation of the MACSQuant Analyzer 10 Flow Cytometer**.
- 6.4 Perform a PMT calibration as described in **SOP 23131 Operation of the MACSQuant Analyzer 10 Flow Cytometer** and document the calibration on **Form 23131-01**.
- 6.5 Adjust the FSC gain to approximately 250 on the X-axis of the FSC vs. SSC plot using a 50 μ L aliquot of the sample to be analyzed as described in **SOP 23131 Operation of the MACSQuant Analyzer 10 Flow Cytometer**.
- 6.6 Perform a spectral compensation as described in **SOP 23131 Operation of the MACSQuant Analyzer 10 Flow Cytometer** using the MACS Comp Bead Kit, anti-REA and 4 μ L (1:50 dilutions) of each antibody used in the compensation. Document the compensation incubation on **Form 23131-01**.
- 6.7 Channels compensated: VioGreen, FITC, PE, PE-Vio770, PI, BLANK

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- 6.8 Prepare Tscm staining solution (TSS) in a 15 mL or 50 mL centrifuge tube by diluting 10% human serum albumin 1:10 (1% final concentration) in Hanks' Balanced Salt Solution (HBSS) and keep cold in the refrigerator (2-8°C) throughout the procedure (Record reagent volumes on **Form 22207-03**). The TSS should be prepared fresh for each experiment. Label the centrifuge tube with TSS, the analyst's initials and the date of preparation.
- 6.9 Store an aliquot of HBSS in the refrigerator (2-8°C) throughout the procedure.
- 6.10 Place an aliquot of cells (up to 1E7 cells) in a microcentrifuge tube and record the input sample volume on **Form 22207-03**.
- 6.11 Dilute the sample 1:2 by adding an equivalent volume of cold HBSS to the aliquot of cells.
- 6.12 Centrifuge at 2,000 rpm (425 xg) for 5 minutes with a slow stop.
- 6.13 Remove the supernatant and wash the cells 2 times with 250 µL of cold HBSS and a 2,000 rpm (425 xg) spin.
- 6.14 Resuspend each cell pellet in 50 µL of cold TSS and add 20 µL of 50 µg/mL Protein L.
- 6.15 Incubate the samples in the refrigerator (2-8°C) for 20 minutes ± 1 minute. Record the incubation start and end times on **Form 22207-03**.
- 6.16 Centrifuge the samples at 2,000 rpm (425 xg) for 5 minutes with a slow stop.
- 6.17 Remove the supernatant and wash each pellet 2 times with 250 µL of cold HBSS and a spin at 2,000 rpm (425 xg) for 5 minutes with a slow stop.
- 6.18 Discard the supernatant from the second wash and resuspend each pellet in 87 µL of cold TSS.
- 6.19 Dilute the anti-Protein L-FITC antibody 1:5 in cold HBSS (Record the reagent volumes on **Form 22207-03**).
- 6.20 Add 5 µL of diluted anti-Protein L-FITC antibody, 2 µL anti-CD45-VioGreen, 2 µL anti-CD33-PE, 2 µL 7-AAD and 2 µL anti-CD3-PE-Vio 770.
- 6.21 Vortex briefly to mix the contents of each tube.
- 6.22 Incubate each sample in the refrigerator (2-8°C) for 20 minutes ± 1 minute. Record the incubation start and end times on **Form 22207-03**.

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- 6.23 Centrifuge the samples at 2,000 rpm (425 xg) for 5 minutes with a slow stop.
- 6.24 Remove the supernatant and wash each pellet 2 times with 250 μ L of cold HBSS and a spin at 2,000 rpm (425 xg) for 5 minutes with a slow stop.
- 6.25 Remove the supernatant and resuspend each cell pellet in an appropriate volume of cold HBSS (typically 200 μ L to 1000 μ L) depending upon the number of cells stained. Record the resuspension volume on **Form 22207-03** as “Post-Staining Sample Volume (μ L)”.
- 6.26 Analyze each sample directly following resuspension using a MACSQuant Analyzer 10 flow cytometer as described below (Section 7).

7. SAMPLE ACQUISITION

- 7.1 Click on File and select New Workspace.
- 7.2 Click on File→ Open...and select Instrument setting on the left side of the Open box.
- 7.3 Browse through the Public, Private or External locations to find the compensation instrument setting prepared in step 6.6.
- 7.4 Select the correct instrument setting and click on Open.
- 7.5 Select the Channels Tab on the sidebar and click on Advanced.
- 7.6 Make sure that the Height box has been selected and press OK.
- 7.7 Click on File→ Open...and select Analysis on the left side of the Open box.
- 7.8 Browse through the Public, Private or External locations to find the proper analysis template.
- 7.9 Select the “Protein L” Analysis template and click on Open.
- 7.10 A warning box will appear indicating that “All existing regions and windows will be removed! Proceed?”. Click on “OK.”
- 7.11 Select the “Experiment” tab on the side toolbar.
- 7.12 Samples may be analyzed one tube at a time by selecting the “Single tube rack” or up to 24 at a time by selecting the “Chill 5 rack” in the Rack drop down menu.

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7.13 Single tube rack

7.13.1 Enter QC Test Request number or a Project name in the Project box.

7.13.2 Enter sample information in the Sample ID and Description boxes.

7.13.3 Select a flow rate and record the flow rate used on **Form 22207-02** as "Flow Rate Used".

NOTE: Use a flow rate that is appropriate for the sample density (Low flow rate for samples that have a high cell density (>1E7), High flow rate for low cell densities (<1E6), Med flow rate for samples with an intermediate cell density).

7.13.4 Select Mix gentle in the Mix sample drop-down menu.

7.13.5 Select Extended in the Mode drop-down menu.

7.13.6 Enter an appropriate volume for analysis in the Uptake volume box (Record volume on **Form 22207-03**) and enter the total sample volume (Post-Staining Sample Volume from **Form 22207-03**) in the Sample volume box.

7.13.7 Select the Annotations tab and modify the annotations for the flow cytometer channels as follows: Channel V2 = CD45-VioGreen, Channel B1 = Protein L-FITC, Channel B2 = CD33-PE, Channel B3 = 7-AAD-PerCP-Vio700, Channel B4 = CD3-PEVio770

7.13.8 Place the tube in the single tube rack and start acquisition by clicking on



the Start Measurement button in the instrument status bar.

7.14 Chill 5 Rack

7.14.1 Enter a Project name or QC Test Request number in the Project box.

7.14.2 Select the wells to be used on the rack window.

7.14.3 Click on Group.

7.14.4 Select a flow rate and record the flow rate used on **Form 22207-03** as "Flow Rate Used".

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- 7.14.5 Select Mix gentle in the Mix sample drop-down menu.
- 7.14.6 Select Extended in the Mode drop-down menu.
- 7.14.7 Select the Annotations tab and modify the annotations for the flow cytometer channels as follows: Channel V2 = CD45-VioGreen, Channel B1 = Protein L-FITC, Channel B2 = CD33-PE, Channel B3 = 7-AAD-PerCP-Vio700, Channel B4 = CD3-PEVio770
- 7.14.8 Select Ungroup.
- 7.14.9 For each tube, enter sample information in the Sample ID and Description boxes for each sample in the Chill 5 rack.
- 7.14.10 For each tube, enter an appropriate volume for analysis in the Uptake volume box (Record volume on **Form 22207-03**) and enter the total sample volume (Post-Staining Sample Volume from **Form 22207-03**) in the Sample volume box.
- 7.14.11 Place the tubes in the Chill 5 rack in the correct locations and start the sample acquisition by clicking on the Start Measurement button in the



instrument status bar.

8. DATA ANALYSIS

- 8.1 The data may be analyzed on the MACSQuant Analyzer 10 instrument or may be copied to an external drive (Scientific Data network location or USB drive) and analyzed on an alternative computer (see **SOP 23131 Operation of the MACSQuant Analyzer 10 Flow Cytometer** for details on how to copy files to an external USB drive).
- 8.2 Click on File and select New Workspace.
- 8.3 Click on the Sample tab.
- 8.4 Right-click and select “Add...”
- 8.5 In the Select Data files to add window, browse through the folders to find the data file to be analyzed, select the file and click on Open.

NOTE: MACSQuant Analyzer 10 data files have a *.mqd extension.

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- 8.6 The flow data may be visualized using the “Protein L” analysis template in either of two ways:
- 8.6.1 Since the “Protein L” analysis template was selected prior to data acquisition (Step 6.9), that analysis template may be applied to the data by going to the Samples tab, right-clicking on the sample name and selecting Apply analysis template.
 - 8.6.2 Alternatively, the “Protein L” analysis template may be applied to the data file by clicking on File → Open. Select Analysis and browse through the folders to find the “Protein L” analysis template and click on Open.
- 8.7 Double-click on each plot to enlarge it and check to ensure that the targeted population of cells is included in the gate.
- 8.7.1 Use the gate edit points to adjust the size and shape of the gate.
 - 8.7.2 Click and drag a gate to move it to a different area of the plot.
- 8.8 Record the percent of CD45+ cells that also express CD3 on **Form 22207-04**, as indicated by %-# on the CD3/CD45 flow plot.
- 8.9 For the Protein L histogram plot, double-click on the plot to enlarge it and adjust the interval gate as needed to include the entire population of Protein L-positive cells as depicted in **Attachment 1**.
- 8.10 Record the Transduction Efficiency Percentage, as indicated by %# on the histogram, on **Form 22207-04**.
- 8.11 Click on Edit and select “Copy plot”.
- 8.12 Paste the histogram into the area indicated on **Form 22207-04**.

9. DOCUMENTATION AND RECORDS

Record all reagent part numbers, lot numbers and expiration dates on **Form 22207-01**. Record the completion of each step on the procedural checklist on **Form 22207-02**. Record the calibration and compensation details on **Form 23131-01**. Record sample preparation details on **Form 22207-03**. Record the results of the assay on **Form 22207-04**.

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10. **REFERENCES AND RELATED DOCUMENTS**

Document Number	Title
22207-01	Protein L Flow Cytometry Assay Reagents
22207-02	Protein L Flow Cytometry Procedural Checklist
22207-03	Protein L Sample Information
22207-04	Protein L Flow Cytometry Results
23131-01	Calibration Compensation

11. **ATTACHMENTS**

Attachment 1 Representative Protein L Histogram Plot

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Attachment 1 Representative Protein L Histogram Plot

