LB2273 Downstream Process Flow Chart

| **Project Information:** |  |
| --- | --- |
| **Project Name** | LB2273 |
| **Lilly Substance Number** | LY4069212 |
| **API Code** | QD563PA |
| **Extinction Coefficient mL/(mg●cm) @ 280nm** | 1.66 |
| **Extinction Coeff. Reference** | The extinction coefficient is calculated based on the Pace method |
| **Clinical Phase** | Phase I |

| **Introduction:** |
| --- |
| This document provides an overview of the operations associated with the downstream processing of LB2273 (LY4069212). The process described will be conducted in the building K360 pilot plant facility located at Lilly Technology Center North (LTC-N), Indianapolis, IN and is being implemented for use in human clinical trials. The process described within this document was developed in BR&D Purification Development, KY242 (LTC-N, Indianapolis) and is being tech transferred to the K360 Pilot Plant for GMP manufacturing.  This process description document provides an operational overview of the downstream purification unit operations used in the manufacture of LB2273 drug substance for use in human clinical trials. The flow diagram below illustrates the sequence of unit operations in the overall LB2273 downstream processing. Each of the unit operations listed are described in the following sections. |

| **Unit Operation** | **Unit Operation Name** | **Item Code** |
| --- | --- | --- |
| **5** | **Detergent Viral Inactivation** | **QD09406** |
| **6** | **Protein A Capture Chromatography** | **QD09407** |
| **7** | **Low pH Viral Inactivation and Clarification** | **QD09408** |
| **8** | **Cation Exchange Chromatography** | **QD09409** |
| **9** | **Viral Filtration** | **QD09411** |
| **10** | **Tangential Flow Filtration** | **QD09412** |
| **11** | **Intermediate Drug Substance Dispensing and Storage** | **QD563PA** |

LB2273 Unit Op 5 Detergent Viral Inactivation (DVI) Process Flow Chart

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| **Unit Operation Purpose:** To inactivate lipid-enveloped viruses using a detergent. | | |
| **Starting Process Intermediate: LY4069212** **Clarified Mammalian Cell Culture (CMCC) Intermediate (QD09405)** | | Estimated titer concentration is 4-5 g/L |
| Operational temperature (°C) | 15 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable | | |

| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| --- | --- | --- | --- |
| **Step 5.1: Adjust CMCC Intermediate to Room Temperature** | | | |
| Temperature (°C) | 15-25 |  |  |
| **Step 5.2: Addition of Detergent** | | | |
| Detergent Composition:  Simulsol SL11W | QD00509 |  |  |
| Detergent addition ratio (grams QD00509 per liter of CMCC Intermediate) | 3.0 |  |  |
| Agitation Time (minutes) | NLT 10 |  | Mixing time of SL11W detergent is based on facility equipment. Reference (1) |
| Final detergent concentration (%w/v) | 0.30 | 0.20-0.40 | Regulatory Commitment (S.2.2). Reference (2) |
| **Step 5.3: Incubation** | | | |
| Agitation | May be continuous |  |  |
| Inactivation Time (minutes) | NLT 180 | NLT 180 | Regulatory Commitment (S.2.2). Reference (2) |
| Temperature (°C) | 15-25 | 15-25 | Regulatory Commitment (S.2.2). Reference (2) |
| **Final Process Intermediate: LY4069212** **Detergent Viral Inactivated Intermediate (QD09406)** | Intermediate Hold Times: (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (3) |
| Approximate expected yield (FIO):  Note: Yield has no Quality impact | 95% |  | Yield loss is due to filtration of process intermediate during transfer and/or assay variability. |
| **References:** | | | |
| 1. RPT-395239, Mixing and Blend Time Assessment for “Green” Detergent Simulsol SL11W as a Potential Replacement for Triton X-100 to Clarified Mammalian Cell Culture Media for Viral Inactivation 2. Control Parameters associated with viral clearance validation. 3. Initial process intermediate hold times based on platform guidance (PRD-00166-TR) | | | |

LB2273 Unit Op 6 Protein A Capture Chromatography (MabSelect PrismA) Process Flow Chart

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| **Unit Operation Purpose:** To capture and purify the LB2273 product from the clarified mammalian cell culture intermediate, including reducing the levels of host cell proteins (HCP) and nucleic acids (DNA). | | |
| **Starting Process Intermediate: LY4069212 Detergent Viral Inactivated Intermediate (QD09406)** | | Estimated titer concentration is 4 - 5 g/L |
| **Chromatography Resin: MabSelect PrismA (QD546S), Mfg.: Cytiva (QD04024 packing heels) (QD09416 used resin)** | | |
| Operational temperature (°C) | 15 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable 2) Chromatography flow velocity may be slowed to accommodate pressure limitations of the equipment set 3) Equipment is configured with a 2 mm flow cell path length | | |

| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| --- | --- | --- | --- |
| **Step 6.1: Column Preparation: Note: All column flow directions are Downflow unless otherwise noted** | | | |
| Column bed height (cm) | 15 – 30 |  |  |
| Flow velocity (cm/hr) | NMT 300 |  |  |
| Pre-use rinse 1 composition: 50 mM Tris, pH 8.0 | QD00015 |  | WFI may be used as sanitization rinse buffer during column packing |
| Pre-use rinse 1 volume (CV) | 0.5 |  |  |
| Pre-use sanitization composition: 500 mM Sodium hydroxide | QD00203 |  | Reference (1,2) Performed prior to first run of each bioreactor. |
| Pre-use sanitization volume (CV) | 2 |  |  |
| Pre-use sanitization total exposure time (min) | 15 - 30 |  | Reference (1) |
| Equilibration buffer composition:  50mM Tris, pH 8.0 | QD00015 |  |  |
| Equilibration volume (CV) | 2 |  |  |
| **Step 6.2: Column Charge** | | | |
| Column load ratio (g/L of resin) | 20 – 40 | 20 - 40 | Regulatory Commitment (S.2.2) |
| Residence Time (min)  or Flow velocity (cm/hr) | NLT 6  NMT 300 |  | Flow is adjusted to meet minimum residence time during load (min). Maximum linear flow velocity (cm/hr) is established due to expected system pressure at higher bed heights. |
| Charge material hold time | (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (3) |
| **Step 6.3: Column Washes** | | | |
| Wash 1 Residence Time (min)  or Flow velocity (cm/hr) | NLT 6  NMT 300 |  | Flow matches load during wash 1 |
| Wash 1 buffer composition:  50mM Tris, pH 8.0 | QD00015 |  |  |
| Wash 1 buffer volume (CV) | 2 |  |  |
| Wash 2 flow velocity (cm/hr) | NMT 300 |  |  |
| Wash 2 buffer composition:  20 mM Tris, 1 M NaCl, pH 7.0 | QD00121 |  |  |
| Wash 2 buffer volume (CV) | 4 |  |  |
| Wash 3 flow velocity (cm/hr) | NMT 300 |  |  |
| Wash 3 buffer composition:  5 mM Sodium citrate, pH 5.5 | QD00514 |  |  |
| Wash 3 buffer volume (CV) | 4 |  |  |
| **Step 6.4: Column Elution** | | | |
| Flow velocity (cm/hr) | NMT 300 |  |  |
| Elution buffer composition:  20 mM Acetic acid, 5 mM Lactic acid | QD00429 |  | Flush bubble trap prior to elution |
| Elution volume (CV) | 5 |  | Next breakpoint allowed after main peak pool complete |
| Frontside MS A280 cutting (AU/cm) | NLT 1.0 | NLT 0.5 | Regulatory Commitment (S.2.2). Mainstream pooling for Viral Clearance studies (if required) is 0.5 A280/cm |
| Backside MS A280 cutting (AU/cm) | NLT 1.0 | NLT 0.5 | Regulatory Commitment (S.2.2). Mainstream pooling for Viral Clearance studies (if required) is 0.5 A280/cm |
| **Final Process Intermediate: LY4069212 Protein A Capture Intermediate (QD09407)** | Intermediate Hold Times: (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (3) Estimated mainstream volume is approximately 1.0-2.0 CV |
| Approximate expected yield (FIO):  Note: Yield has no Quality impact | 90 % |  | Greater than or equal to 90%  Ref: eb86867-2024-0012 and eb86867-2025-0006 |
| **Step 6.5: Column Regeneration** | | | |
| Flow velocity (cm/hr) | NMT 300 |  |  |
| Regeneration composition: 20 mM Acetic acid, 5 mM Lactic acid | QD00429 |  |  |
| Regeneration volume (CV) | 3 |  |  |
| **Step 6.6: Column Sanitization** | | | |
| Sanitization flow direction | Upflow |  |  |
| Rinse 1 composition: 50 mM Tris, pH 8.0 | QD00015 |  | WFI may be used as sanitization rinse buffer during column packing |
| Rinse 1 volume (CV) | 0.5 |  |  |
| Sanitization composition: 500 mM Sodium hydroxide | QD00203 |  | Reference (1) Sanitization performed after every cycle. 1N NaOH may be used post-packing or in response to elevated microbial levels. Reference (4) |
| Sanitization volume (CV) | 2 |  |  |
| Sanitization total exposure time (minutes) | 15 - 30 |  | Reference (1) |
| **Step 6.7: Column Storage** | | | |
| Rinse 2 composition: 50 mM Tris, pH 8.0 | QD00015 |  | WFI may be used as sanitization rinse buffer during column packing |
| Rinse 2 volume (CV) | 0.5 |  |  |
| Flow velocity (cm/hr) | NMT 300 |  |  |
| Storage flow direction | Upflow |  |  |
| Storage composition: 100 mM Acetic acid / sodium acetate, pH 4.0 | QD00249 |  | Column storage is performed if the column will be held > 24 hours prior to the next cycle |
| Storage volume (CV) | 2 |  | pH endpoint of NMT 4.5 |
| **References:** | | | |
| 1. Resin Management in K360 Pilot Plant Operations (PRD-01741-TR) 2. Microbial Challenge Data Establish Acceptable Storage Times for Chromatography Columns without Requisite Re-Sanitization (TR2009OCT01\_1025A009286) 3. Initial process intermediate hold times based on platform guidance (PRD-00166-TR) 4. Strip Cleaning and Microbial Sanitization Process Recommendations for Next Generation / Caustic Stable Protein A Chromatography Resins, Brian Youchak., et al. (RPT-957831). | | | |

A graph of a graph

AI-generated content may be incorrect.Representative chromatogram from eb86867-2024-0024

LB2273 Unit Op 7 Low pH Viral Inactivation and Clarification Process Flow Chart

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| **Unit Operation Purpose:** 1) To inactivate potential viral contaminants sensitive to low pH exposure. 2) To reduce trace impurities of CHO host cell proteins (HCP). 3) To provide a neutralized LB2273 (LY4069212) process intermediate appropriate for further processing. | | |
| **Starting Process Intermediate: LY4069212 Protein A Captured Intermediate (QD09407)** | | Estimated A280 protein concentration is 10 - 33 g/L |
| **Depth Filter: Millipore X0SP POD** | | |
| Operational temperature (°C) | 15 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable | | |

| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| --- | --- | --- | --- |
| **Step 7.1: Adjust to Target pH (3.45)** | | | |
| Low pH titrant composition: 50 mM HCl | QD00496 |  |  |
| Volume of titrant (L) | determined by offline titration |  | Estimated to be approximately 0.2 – 0.3 liters of titrant per liter of ProA-MS (eLN eb86867-2024-0013 and eb86867-2025-0007) |
| Order of addition | add titrant to ProA-MS |  |  |
| Mix to homogeneity |  |  | Facility specific |
| Adjusted pH (after addition) | 3.45 (3.30 – 3.60) | NMT 3.60 | Regulatory Commitment (S.2.2) |
| **Step 7.2: Low pH Viral Inactivation** | | | |
| Temperature (oC) | 20 (18 – 25) | NLT 18 | Regulatory Commitment (S.2.2) |
| Time (minutes) | NLT 180 | NLT 180 | Regulatory Commitment (S.2.2) |
| pH (at end of inactivation time) | NMT 3.60 | NMT 3.60 | Regulatory Commitment (S.2.2) |
| **Step 7.3: Neutralization** | | | |
| Neutralization titrant composition: 250 mM Tris Base | QD00156 |  |  |
| Volume of titrant (L) | determined by offline titration |  | Estimated to be approximately 0.08 liters of titrant per liter of LpH-VI (eLN eb86867-2025-0007) |
| Order of addition | add titrant to LpH-VI |  |  |
| pH (after neutralization) | 7.00 (6.70 – 7.30) |  |  |
| Mixing time after reaching target pH (minutes) | NLT 15 |  |  |
| **Step 7.4: Depth Filtration (Millipore X0SP)** | | | |
| Continue agitation during filtration |  |  |  |
| Flush target flux (LMH) | 300 |  | If target flux value cannot be achieved, a flow rate of 10 L/min will be used |
| Filter flush composition: WFI | QA119A |  |  |
| Filter flush volume (L/m2) | NLT 50 |  |  |
| Depth Filtration target flux (LMH) | NMT 200 |  |  |
| Depth filter load ratio (g/m2) | NMT 1000 |  | 884 g/m2 tested eLN eb86867-2025-0007 |
| Post-use depth filter flush volume (L/m2) WFI | 20 |  | Corresponds to approximately 2 internal void volumes for this filter |
| **Step 7.5: CEX Charge Preparation** | | | |
| Titrant composition: 0.1 N acetic acid | QD00224 |  |  |
| Volume of titrant (L) | determined by offline titration |  | Estimated to be approximately 0.094 liters of titrant per liter of X0SP filtrate (eLN eb86867-2025-0007) |
| Order of addition | add titrant to X0SP filtrate |  |  |
| Final pH | 5.00 (4.70 – 5.30) |  |  |
| **Final Process Intermediate: LY4069212 Neutralized Low pH Viral Inactivated Intermediate (QD09408)** | Intermediate Hold Times: (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (1) |
| Approximate expected yield (FIO):  Note: Yield has no Quality impact | 90 % |  | eLN reference for expected yield: eb8687-2025-0007 |
| **References:** | | | |
| 1. Initial process intermediate hold times based on platform guidance (PRD-00166-TR) | | | |

LB2273 Unit Op 8 Cation Exchange Chromatography (Poros 50 HS) Process Flow Chart

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| **Unit Operation Purpose:** To further purify the LB2273 (LY4069212) product bispecific antibody and to reduce the levels of aggregates and host cell proteins (HCP) | | |
| **Starting Process Intermediate: LY4069212 Neutralized Low pH Viral Inactivated Intermediate (QD09408)** | | Estimated A280 protein concentration is 13 - 16 g/L |
| **Chromatography Resin: Poros 50 HS (QD515E), Mfg.: Life Technologies (QD04015 packing heels) (QD09415 used resin)** | | |
| Operational temperature (°C) | 15 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable 2) Chromatography flow velocity may be slowed to accommodate pressure limitations of the equipment set 3) Equipment is configured with a 2 mm flow cell path length | | |

| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| --- | --- | --- | --- |
| **Step 8.1: Column Preparation: Note: All column directions are Downflow unless otherwise noted** | | | |
| Column bed height (cm) | 15 – 30 |  |  |
| Flow velocity (cm/hr) | NMT 300 |  |  |
| Pre-use sanitization composition: 1M Sodium hydroxide | QD00007 |  | Reference (1,2) Prior to first run of each bioreactor |
| Pre-use sanitization volume (CV) | 2 |  |  |
| Pre-use sanitization total exposure time (min) | NLT 30 |  | Reference (1). Flow velocity is adjusted or stopped to achieve exposure time |
| Pre-Equilibration buffer composition:  20 mM Sodium acetate, 500 mM NaCl, pH 5.0 | QD00346 |  |  |
| Pre-Equilibration volume (CV) | 2 |  |  |
| Equilibration buffer composition:  20 mM Sodium acetate, pH 5.0 | QD00305 |  |  |
| Equilibration volume (CV) | 3 |  | Manual advance if stable for ~2CV |
| **Step 8.2: Column Charge** | | | |
| Column load ratio (g/L of resin) | 15 - 25 | 15 - 25 | Regulatory Commitment (S.2.2) |
| Charge flow velocity (cm/hr) | NMT 200 |  |  |
| **Step 8.3: Column Wash** | | | |
| Wash flow velocity (cm/hr) | NMT 200 |  |  |
| Wash buffer composition: | 80% A: QD00305 20% B: QD00346 |  | Column off-line until flow ratio is established |
| Wash volume (CV) | 2 |  |  |
| **Step 8.4: Column Elution** | | | |
| Elution flow velocity (cm/hr) | NMT 200 |  |  |
| Elution linear gradient Start composition: | 80% A: QD00305 20% B: QD00346 |  |  |
| Linear gradient volume (CV) | 12.5 |  | Estimated mainstream volume is approximately 2.5-3.5 CV. |
| Linear gradient End composition: | 30% A: QD00305 70% B: QD00346 |  |  |
| Isocratic hold composition:  **A:** 20 mM Sodium acetate, pH 5.0 **B:** 20 mM Sodium acetate, 500 mM NaCl, pH 5.0 | 30% A: QD00305 70% B: QD00346 |  | If necessary to complete elution |
| Isocratic hold volume (CV) | 2 |  |  |
| Frontside MS A280 cutting (AU/cm) | NLT 3.0 | NLT 3.0 | Regulatory Commitment (S.2.2) |
| Backside MS A280 cutting (AU/cm) | NLT 4.0 | NLT 4.0 | Regulatory Commitment (S.2.2) |
| **Final Process Intermediate: LY4069212 Cation Exchange Intermediate (QD09409)** | Intermediate Hold Times: (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (3) |
| Approximate expected yield (FIO):  Note: Yield has no Quality impact | 75 - 80% |  | eLN reference for expected yield: eb86867-2025-0008 |
| **Step 8.5: Column Regeneration** | | | |
| Regeneration flow velocity (cm/hr) | NMT 300 |  |  |
| Regeneration flow direction | Upflow |  |  |
| Regeneration composition: 20 mM Sodium acetate, 500 mM NaCl, pH 5.0 | QD00346 |  | Post-use regeneration/sanitization is performed after every chromatography cycle |
| Regeneration volume (CV) | 2 |  |  |
| **Step 8.6: Column Sanitization** | | | |
| Sanitization flow velocity (cm/hr) | NMT 300 |  |  |
| Sanitization flow direction | Upflow |  |  |
| Regeneration/ Sanitization composition: 1 M Sodium hydroxide | QD00007 |  | Post-use regeneration/sanitization is performed after every chromatography cycle |
| Sanitization volume (CV) | 2 |  |  |
| Sanitization total exposure time (min) | NLT 30 |  | Reference (1). Flow velocity is adjusted or stopped to achieve exposure time |
| **Step 8.7: Column Storage** | | | |
| Storage flow velocity (cm/hr) | NMT 300 |  |  |
| Storage flow direction | Upflow |  |  |
| Storage composition: 0.01 N NaOH | QD00008 |  | Column storage is performed if the column will be held > 24 hours prior to the next cycle |
| Storage volume (CV) | 2 |  |  |

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| **References:** |
| 1. Resin Management in K360 Pilot Plant Operations (PRD-01741-TR) 2. Microbial Challenge Data Establish Acceptable Storage Times for Chromatography Columns without Requisite Re-Sanitization (TR2009OCT01\_1025A009286) 3. Initial process intermediate hold times based on platform guidance (PRD-00166-TR) |

**A graph of a graph

AI-generated content may be incorrect.**

Representative chromatogram from eb86867-2025-0008 (25 g/Lr load)

LB2273 Unit Op 9 Viral Filtration (Planova 20N) Process Flow Chart

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| **Unit Operation Purpose:** To remove putatively present viral particles by nano-filtration | | |
| **Starting Process Intermediate: LY4069212 Cation Exchange Intermediate (QD09409)** | | Estimated A280 protein concentration is 4 – 6 g/L |
| **Virus Filter: Planova 20N (CN0738 4.0m2), Mfg.: Asahi Kasei** | | |
| Operational temperature (°C) | 15 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable | | |

| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| --- | --- | --- | --- |
| **Step 9.1: Membrane Preparation:** | | | |
| Pre-use flush composition: WFI | QA119A |  |  |
| Pre-use air leak test pressure (psig) 0.22 mm Filtered process air | 13.5 – 14.9 |  | Range based on manufacturer (Asahi) recommendation and literature. Modular viral clearance package supports maximum pressure up to 16 psig. |
| Buffer flush composition 20 mM Sodium acetate, pH 5.0 | QD00305 |  |  |
| Tube side buffer flush pressure (psig) | 2.8 – 4.3 |  |  |
| Tube side buffer flush volume (L/m2) | NLT 1 |  |  |
| Filtrate buffer flush pressure (psig) | 12.0 – 16.0 |  |  |
| Filtrate buffer flush volume (L/m2) | NLT 3 |  |  |
| **Step 9.2: Product Pre-Filtration** | | | |
| Pre-Filter | 0.22 mm |  |  |
| **Step 9.3: Viral Filtration** | | | |
| Membrane volumetric loading capacity (L/m2) | NMT 140 | NMT 150 | Regulatory Commitment (S.2.2). pCPP value not finalized until lab-scale viral clearance studies are performed post initial CT manufacturing campaign. |
| Viral filtration pressure (psig) AI90 | 12.0 – 16.0 | 12.0 – 16.0 | Regulatory Commitment (S.2.2). Reference (1,2). Lower pressure limit must be met within 10 minutes of product filtration start. Complete product filtration in one transfer without de-pressurizing filter until all product is filtered. |
| **Final Process Intermediate: LB2273 Viral Filtration Intermediate (QD09411)** | Intermediate Hold Times: (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (3) |
| Approximate expected yield (FIO):  Note: Yield has no Quality impact | 98 - 100% |  | Any yield loss is due to filtering of process intermediate or assay variability. |
| **Step 9.4: Post-Use Filter Integrity Test** | | | |
| Post-use air leak test pressure (psig) 0.22 mm Filtered process air | 13.5 – 14.9 |  | Range based on manufacturer (Asahi) recommendation and literature. Modular viral clearance package supports maximum pressure up to 16 psig. |
| Cleaning solution composition: 0.25M NaOH, 1% (w/v) SDS | QD00540 |  |  |
| Cleaning pressure (psig) | NMT 11.4 |  |  |
| Tube side cleaning volume (L/m2) | NLT 0.5 |  |  |
| Filtrate cleaning volume (L/m2) | NLT 1 |  |  |
| WFI flush pressure (psig) | NMT 11.4 |  |  |
| WFI tube side flush volume (L/m2) | NLT 0.5 |  |  |
| WFI filtrate flush volume (L/m2) | NLT 3 |  |  |
| Gold particle integrity test pressure (psig) | 3.5 – 4.3 |  |  |
| Gold particle integrity test volume (L/m2) | 1 |  |  |
| LRV | ≥ 1.40 | PASS | Regulatory Commitment (S.2.2). Reference (1). If integrity test indicates a failing result, the process solution must be re-processed through a new viral filter. |
| **References:** | | | |
| 1. Control parameters associated with modular viral clearance package for Planova 20N (PRD-00698-TR) 2. Laboratory data suggests that de-pressurization and re-pressurization during product filtration causes a reduced parvovirus log reduction factor (eLN EL01278-025) “Impact of de-pressurization / re-pressurization during Planova 20N filtration process on parvovirus retention” 3. Initial process intermediate hold times based on platform guidance (PRD-00166-TR) | | | |

LB2273 Unit Op 10 Tangential Flow Filtration Process Flow Chart

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| **Unit Operation Purpose:** To exchange the LB2273 (LY4069212) product into the appropriate buffer for final drug substance (DS) preparation and concentrate the product to the appropriate range for final DS preparation. | | |
| **Starting Process Intermediate: LY4069212 Viral Filtered Intermediate (QD09411)** | | Estimated protein concentration is 4 - 6 g/L |
| **TFF Membrane: Millipore 30 kDa Pellicon 3 Ultracel Regenerated Cellulose D screen** | | |
| Operational temperature (°C) | 2 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable | | |

| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| --- | --- | --- | --- |
| **Step 10.1: Membrane Preparation** | | | |
| **Initial Membrane Preparation:** | | | |
| Recirculation flow rate (L/min/m2) | 5, 2 - 6 |  |  |
| Water flush WFI | QA119A |  |  |
| Water flush volume (L/m2) | 60 |  |  |
| Integrity test pressure (psig) | 30 |  | Air permeability test will be executed on the first run only (new membranes or used membranes that are re-installed) |
| Integrity test air flow (mL/min/m2) | NMT 103 |  |
| Sanitization buffer composition: 0.1 N NaOH | QD00009 |  | Sanitization performed on the first run of each bioreactor |
| Sanitization time (minutes) | NLT 30 |  | Reference (1) |
| **Membrane Preparation:** | | | |
| Water flush time (minutes) | NLT 15 |  |  |
| Equilibration buffer composition: 10 mM histidine, 8% sucrose, pH 5.75 | QD00562 |  |  |
| Equilibration time (minutes) | NLT 15 |  |  |
| **Step 10.2: Primary Concentration** | | | |
| Recirculation flow rate (L/min/m2) | 5, 2 - 6 |  |  |
| TMP (psig) | 15 - 25 |  |  |
| Membrane load (g/m2) | NMT 1000 |  |  |
| Target concentration (g/L) | 60, 50 - 70 |  |  |
| **Step 10.3: Diafiltration** | | | |
| Diafiltration buffer composition: 10 mM histidine, 8% sucrose, pH 5.75 | QD00562 |  |  |
| Recirculation flow rate (L/min/m2) | 5, 2 - 6 |  |  |
| TMP (psig) | 15 - 25 |  |  |
| Diafiltration buffer volumes | 8, NLT 7 | NLT 7 | Regulatory Commitment (S.2.2) |
| **Step 10.4: Secondary Concentration** | | | |
| Recirculation flow rate (L/min/m2) | 0.5 - 6 |  |  |
| Inlet pressure (psig) | NMT 70 |  |  |
| Target concentration (g/L) | 60. 50 -70 |  |  |
| **Step 10.5: Product Recovery and Dilution** | | | |
| Flush buffer composition: 10 mM histidine, 8% sucrose, pH 5.75 | QD00562 |  |  |
| Flush volume | TBD |  | Flush volume to be determined by on-the-floor protein concentration reading |
| Protein concentration (g/L) | 50, 46 - 54 | 45 - 55 | Regulatory Commitment (S.2.2). |
| pH | 5.8, 5.4 – 6.2 | 5.3 – 6.3 | Regulatory Commitment (S.2.2). |
| **Step 10.6: Filtration** | | | |
| Filter membrane porosity (mm) | 0.2 |  |  |
| **Final Process Intermediate: LY4069212 TFF Intermediate (QD09412)** | Intermediate Hold Times: (15-25oC) NMT 48 hrs (2-8oC) NMT 7 days | | Reference (2) |
| TFF Intermediate Density | 1.043 g/mL |  | Notebook eb86867-2024-0010 |
| Approximate expected yield (FIO):  Note: Yield has no Quality impact | 98 % |  | Notebook eb86867-2025-0010 |
| **Step 10.7: TFF Membrane Cleaning and Sanitization** | | | |
| Cleaning/Sanitization frequency | After every cycle | |  |
| Recirculation flow rate (L/min/m2) | 5, 2 - 8 |  |  |
| Flush buffer composition: 10 mM histidine, 8% sucrose, pH 5.75 | QD00562 |  |  |
| Flush buffer volume (number of hold-up volumes) | NLT 2 |  |  |
| Cleaning/Sanitization buffer composition: 0.1 N NaOH | QD00009 |  |  |
| Cleaning/Sanitization volume (number of hold-up volumes) | NLT 2 |  |  |
| Cleaning/Sanitization time (minutes) | NLT 60 |  | Reference (1) |
| For membrane re-use proceed to membrane equilibration above | | | |
| **Step 10.8: TFF Membrane Storage** | | | |
| Storage frequency | If membranes held >24 hours prior to next cycle | |  |
| Recirculation flow rate (L/min/m2) | 5, 2 - 6 |  |  |
| Storage composition: 0.1 N NaOH | QD00009 |  |  |
| Storage flush time (minutes) | NLT 2 |  |  |
| Membrane storage time | NMT 6 months (NMT 30oC) NMT 2 years if storage solution is changed every 6 months | |  |
| **References:** | | | |
| 1. Microbial Challenge Data Establish Acceptable Sanitization Times for Chromatography and TFF Systems (TR2009SEP30\_0658A009286) 2. Initial process intermediate hold times based on platform guidance (PRD-00166-TR) | | | |

LB2273 Unit Op 11 LB2273 `

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| **Unit Operation Purpose:** To add polysorbate 80 to complete the DS formulation.  Filter and Dispense DS into an approved container closure system for storage and transport at NMT -65°C prior to drug product manufacture. | | |
| **Starting Process Intermediate: LY4069212 Tangential Flow Filtered Intermediate (QD09412)** | | Estimated protein concentration is 50 g/L |
| Operational temperature (°C) | 15 - 25 | Applicable to all steps unless otherwise stated |
| **Notes:**  1) When only a target value is provided for a parameter (no stated range or criticality), a variation of +/- 10% around the target value is allowable | | |

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| **Process Parameter** | **Operating Range** | **pCPP** | **Notes/Reference** |
| **Step 11.1:Polysorbate Addition** | | | |
| Polysorbate 80 composition, 10% | QD00023 |  |  |
| Add polysorbate to TFF– final concentration (w/v) | 0.05% |  |  |
| **Step 11.2: Filtration** | | | |
| Filter membrane porosity (mm) | 0.2 |  |  |
| **Step 11.3: Dispensing** | | | |
| Container | Polycarbonate (PC) Bottles |  |  |
| Bulk Aliquot Volume | Target 5000mL (340mL – 5100mL)  Target 1000mL (150mL – 1050mL) |  |  |
| **Step 11.4: Storage** | | | |
| Final API Intermediate Density | 1.043 g/mL |  | Reference (1) |
| Storage Temperature | NMT -65C long term |  |  |
| **Final Process Intermediate: LY4069212 (LB2273) API Intermediate (QD563PA)** | | | |
| **References:** | | | |
| 1. Density measured in NuGenesis notebook eb86867-2024-0016 (TFF-F at 50 g/L) and eb86867-2025-0011 (API at 48 g/L) for LB2273. Density Value is representative of this API intermediate. | | | |

**Reasons for Revision**

| **Version** | **Step** | **Reasons for Revision** |
| --- | --- | --- |
| **1** | All | Original document |
| **2** | All | Update item numbers |
| **3** | 10.2 | Membrane load level changed from NMT 500g/m2 to NMT 1000g/ m2 |