Batch Record: Anti-IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point.

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2017 use 10JUL17.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: Anti-IL-8 mAb Production from CHO DP-12 Cells
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1. Initial Media Preparation and Media hold		
1.1 Clean, assemble and autoclave one 100 mL Bellco Spinner flask per SOP.	Operator/Date	Verifier/Date
1.2. Obtain sterile 50 mL conical tube.	Operator/Date	Verifier/Date
1.3. Obtain sterile DMEM Media. Manufacturer:_____Catalog number: _____ Lot number:_____Expiration date:_____	Operator/Date	Verifier/Date
1.4. Obtain sterile Super Low Fetal Bovine Serum (FBS). Manufacturer:_____Catalog number: _____ Lot number:_____Expiration date:_____	Operator/Date	Verifier/Date
1.5. Obtain sterile 100X Insulin-Transferrin Selenium (ITS-G). Manufacturer:_____Catalog number: _____ Lot number:_____Expiration date:_____	Operator/Date	Verifier/Date
1.6. Obtain sterile 1000X methotrexate solution (2mM). Manufacturer:_____Catalog number: _____ Lot number:_____Expiration date:_____	Operator/Date	Verifier/Date
1.7. Aseptically prepare **120 mL** Complete Growth Media – DMEM/10% FBS, 1X ITS-G, 200nM methotrexate.		
1.7. 1 **Obtain** 250 mL **of** 0.22uM sterile filter unit. Manufacturer:_____Catalog number: _____ Lot number:_____Expiration date:_____ 1.7.2. Transfer the following to the top portion of the filter top unit and sterile filter: • 107 mL of DMEM media • 12 mL of Superlow IgG FBS • 1.12 mL of 100X ITS-G • 0.12 mL of 1000X methotrexate (2mM) 1.7.3. Label the media bottle “CHO DP12 Complete Growth Media”.	Operator/Date	Verifier/Date
1.8. 1. Label spinner flask as CHO DP12, [date], [group#], [operator initials]. **1.8.2. Label 50 mL** conical tube as “Blank”.	Operator/Date	Verifier/Date

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1.9 Aseptically transfer 98 mL of Complete Growth Media to 100 mL spinner flask. 100mL spinner flask ID# _____ Vol of Complete Growth Media _____	Operator/Date	Verifier/Date
1.10. Aseptically transfer 20 mL of Complete Growth Media to 50 mL conical tube. 50 mL conical tube ID # _____ Vol of Complete Growth Media _____	Operator/Date	Verifier/Date
1.11.1 Place spinner flask and 50 mL tube containing CHO Complete Growth Media in the CO_2 incubator. Make sure to loosen side arm caps of spinner flask once in incubator. **1.11.2** Set the speed of the magnetic stirrer to 60 rpm setting.	Operator/Date	Verifier/Date
1.13. Verify that CO_2 is set to 5±0.5% and that temperature is set to 37±0.5ºC. CO_2_____% Temperature_____ ºC	Operator/Date	Verifier/Date
1.14. Check media for contamination after a minimum of 24 hrs. Incubation start time:_____ Incubation end time: _____ Elapsed time: _____ 100mL spinner flask ID_____Contamination: Y / N (Circle) 50 mL tube ID_____Contamination: Y / N (Circle)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: Anti-IL-8 mAb Production from CHO DP-12 Cells
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2. Inoculation of Spinner Flasks		
2.1. Remove two vials of CHO cells from storage in the -80°C freezer. Vial ID _____ Vial ID _____ Cell Concentration _____ Cell Concentration _____ Cryopreservation date _____ Cryopreservation date _____	Operator/Date	Verifier/Date
2.2. Thaw vials contents rapidly by agitation in a 37°C ± 0.5°C water bath. Water bath temperature: _____	Operator/Date	Verifier/Date
2.3. Aseptically transfer the entire contents of each 1 mL vial of thawed CHO cells into the previously prepared Spinner Flask containing 98 mL CHO Complete Growth Medium using a 1 mL sterile pipette. **Swirl** to mix. **Do not add any CHO Cells to the 50 ml conical tube labeled "Blank".**	Operator/Date	Verifier/Date
2.4.Transfer the spinner flask and the 50 mL conical tube to the CO_2 incubator at 37ºC with 5% CO_2. **Verify** that CO_2 is set to 5±0.5% and that temperature is set to 37±0.5ºC. CO_2___% Temperature___ ºC	Operator/Date	Verifier/Date
2.5. Place spinner flask on magnetic stirrer in the CO_2 incubator. Make sure to loosen side arm caps of spinner flask once in incubator. Set stirrer for 60 rpm for 15 minutes ± 5 minutes and take Day 0 sample.	Operator/Date	Verifier/Date
3. Monitoring of Spinner flask Cell Culture. Immediately after inoculation of the bioreactor (Day 0) and at 1 - day intervals, sample the culture to determine OD at 650 nm, viable cell count and viability, concentration of glucose, concentration of lactate, and anti-IL-8 concentration. Once the cell concentration of the cell culture reaches ≥ 1,000,000 cells/mL the cell culture is scaled up to 1 L bioreactor.		
3.1 **Label** 5 microfuge tubes as follows: "cells", "cell count", "trypan blue" ,"balance", "anti-IL-8-vessel name, day of culture, group initials date". 3.1.2. Label 2 spectrophotometer cuvettes as follows: "blank" and "sample" 3.1.3. Swab and take 15 mL conical tube in the BSC and label as "anti IL-8, date, initials"	Operator/Date	Verifier/Date

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3.2. 1. In BSC, aseptically remove 1 mL of blank media and place in microfuge tube labelled "blank". 3.2.2. Aseptically remove 3 mL of cell suspension from spinner flask and place in labeled 15 mL conical tube.	Operator/Date	Verifier/Date
3.3. Return CHO DP-12 labeled spinner flask and blank 50 mL conical tube to the CO_2 incubator**,** making sure to loosen arm caps of spinner flask.	Operator/Date	Verifier/Date
3.4.1. Remove 15 mL conical tube containing 3 mL sample conical from the BSC. 3.4.2. Remove microfuge tube containing 1 mL blank from the BSC.	Operator/Date	Verifier/Date
3.5 Cell Viability 3.5.1**.** Remove 100µL from 15 mL conical tube containing 3 mL sample and place in microfuge tube labeled "cells count". 3.5.2. Determine viable cell count per Trypan Blue Assay SOP. Record cell viability and concentration in the table on page 7 of the Batch Record.	Operator/Date	Verifier/Date
3.6. pH measurement 3.6.1 Calibrate pH Meter per Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP. 3.6.2. Using the remaining 2.9 mL sample in the 15 mL conical tube take the pH reading per Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP. Record pH in the table on page 7 of the Batch Record.	Operator/Date	Verifier/Date
3.7. OD 650 measurement 3.7.1**.** Prepare spectrophotometer per SOP. Using the same 2.9 mL sample from step 3.5, transfer 1 mL of sample to the cuvette labeled "sample". Pipet the sample up and down in the cuvette several times to mix. 3.7.2. Transfer the 1 mL of blank media from the microfuge tube labeled "blank" into cuvette labeled "blank". Measure OD reading at 650 nm per Spectrophotometer SOP. Blank the spectrophotometer using the cuvette with the blank. Record the O.D in the table on page 7 of the Batch Record.	Operator/Date	Verifier/Date

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3.8. Measurement of Glucose, Lactate, and Anti- IL-8 concentration 3.8.1. Remove 1 mL of the remaining 1.9 mL sample and place in the microfuge tube labelled "cells". Place 1 mL of millQ water in microfuge tube labeled "balance". Centrifuge both "cells" and "balance tubes" for 5 minutes in microcentrifuge 3.8.1. Remove supernatant from the sample tube and transfer to microfuge tube labeled "anti- IL-8 vessel name (Spinner or Bioreactor), day of culture, group initials, date". 3.8.2. Store sample at 2-8°C in microfuge storage box labeled with date, group name, for measurement of glucose, lactate, and anti-IL8 concentration. 3.8.3. Add 10% bleach solution to the remaining sample and discard in the biohazard waste.	Operator/Date	Verifier/Date
When the 100 mL suspension culture of CHO cells reaches a concentration of $\geq 1 \times 10^6$ cells/mL, the entire contents of the spinner flask will be added to the bioreactor containing 1L of CHO cell growth media.		
Comments	Operator/Date	Verifier/Date

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100 mL Spinner Flask ID# ___________________________________

TIME (hours)	**OD 650nm**	**pH**	**Viable cells/mL**	**Percent Viability**	**GLUCOSE (mg/dL)**	**LACTATE (mmol/L)**
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: Anti-IL-8 mAb Production from CHO DP-12 Cells
Lot Number ___________________________

100 mL Spinner Flask ID# __________________________________

TIME (hours)	**OD 650nm**	**pH**	**Viable cells/mL**	**Percent Viability**	**GLUCOSE (mg/dL)**	**LACTATE (mmol/L)**
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

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Bioreactor Scale Up		
4. Buffer Preparation 1M ($NaHCO_3$) sodium bicarbonate- 150mL 1X PBS Phosphate buffered Saline- 100 mL		
4.1 Preparation of 1M NaHC03 **4.1.1 Label** 500 mL glass feed bottle 1M$NaHCO_3$, [date], [initials], and storage: room temperature, disposal; drain. **4.1.2 Weigh** out 12.6. ± 0.1 grams of ($NaHCO_3$) sodium bicarbonate and transfer to a 250 mL beaker Balance ID _____ NaHC03 manufacturer _____ Catalog number _____ Lot number _____ Expiration Date _____ **4.1.3** Using a 250 mL graduated cylinder, measure 100mL MilliQ water and add to the NaHCO3 in the beaker Volume of MilliQ water added _____ mL **4.1.4**. Add magnetic stir bar and stir on a magnetic stirrer to dissolve. Transfer dissolved NaHCO3 to a 250 mL graduated cylinder and bring to the volume at 150 mL with MilliQ water. Transfer 150 mL 1M NaHC03 to labeled alkaline feed bottle **4.1.5.** Prepare labeled alkaline feed bottle for autoclave. Cover the bottle and tubing per Applikon Bioreactor Controller SOP.	Operator/Date	Verifier/Date

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4.2. Prepare 1X PBS 4.2.1 In a 100 mL graduated cylinder, add 10 mL 10X PBS and bring to volume with 90 mL MilliQ water 10X PBS: Manufacturer:_____ Catalog number: _____ Lot number:_____ Expiration date:_____ Volume of 10X PBS added:_____mL Volume of water added:_____mL 4.2.2. Store the prepared 1X PBS in a bottle labelled 1X PBS, [date],[initials]	Operator/Date	Verifier/Date
5. Prepare the controller as per the Applikon Bioreactor Controller Operation SOP step 8.1	Operator/Date	Verifier/Date
6. Assemble/Autoclave Bioreactor		
6.1. Assemble the Vessel stand if not assembled	Operator/Date	Verifier/Date
6.2. Inspect the integrity of the large O- rings on the vessel stand and headplate. Replace if worn or cracked. Bioreactor ID #_____ Vessel stand O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Head plate O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
6.3 Assemble Head plate Underside		
6.3.1. Inspect the integrity of the O-rings on the harvest tube, sparger, and the thermowell. Harvest tube O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Sparger O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Thermowell O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
6.3.2. Attach sample tube, sparger and thermowell. **Verify** that the sparger tube is aligned beneath the stirrer impeller.	Operator/Date	Verifier/Date

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6.3.3 Add 100 mL of 1X PBS to the bioreactor.	Operator/Date	Verifier/Date
6.4. Attach head plate to Vessel Stand.		
6.4.1. Place the head plate onto the vessel stand, positioning the holes on the outer edge of the head plate with the bolts on the vessel stand. **Secure** the head plate with the 5mm fasteners.	Operator/Date	Verifier/Date
6.5 Assemble Head plate – Topside		
6.5.1. Inspect the integrity of the O-ring in the condenser port of the head plate. Replace if worn or cracked. Condenser port O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) **6.5.2. Inspect** the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked. **6.5.3. Attach** the condenser to the head plate by placing the condenser into the condenser port making sure that the barbed connectors are facing out.	Operator/Date	Verifier/Date
6.6. DO Probe Preparation		

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6.6.1. Remove the protective cap from the bottom of the stainless steel DO probe. **6.6.2. Inspect** the screen at the bottom of the probe tip. Replace if damaged. **6.6.3. Unscrew** the membrane module from the bottom housing of the probe tip by holding the probe in the vertical position. Inspect the integrity of the O-ring. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one) O-ring replaced? Yes / No (Circle one) **6.6.4. Replenish** DO electrolyte. There should be 1 mL of O_2 electrolyte solution in the membrane module. Screw the membrane module. **6.6.5. Inspect** the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one) O-ring replaced? Yes / No (Circle one) **6.6.6 Inspect** the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one) Black seal replaced? Yes / No (Circle one) **6.6.7. Attach** DO probe to the head plate.	Operator/Date	Verifier/Date
6.7. Calibrate the pH probe per Applikon Bioreactor Controller Operation SOP. (Refer step 8.2.2.)		
6.7.1. Obtain pH 7 and pH 4 calibration buffers. pH 7 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____ pH 4 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____	Operator/Date	Verifier/Date

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6.7.2. Perform 2 point calibration per Applikon Bioreactor Controller Operation SOP (Refer step 8.2.2). **Record** pH calibration values. pH 7.00 standard: pH value_____temp_____ pH 4.00 standard: pH value_____temp_____ Slope from the Display _____Expected value: 0.95-1.05 Offset from the Display_____Expected value: < ±0.3	Operator/Date	Verifier/Date
6.7.3 Inspect the integrity of the O-ring at the top of the pH probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
6.7.4.. Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
6.7.5. Attach pH probe to the head plate.	Operator/Date	Verifier/Date
6.8. Preparation of liquid addition bottles and attaching the filters and tubing per the Applikon Bioreactor Controller Operation SOP.		
6.8.1 Verify that the liquid addition bottles are prepared as per the 8.2.4 section of the Applikon Bioreactor Controller Operation SOP for preparing the bioreactor.	Operator/Date	Verifier/Date
6.8.2. Mount the connections to the bioreactor by following steps listed in the section 8.2.5 in the Applikon Bioreactor Controller Operation SOP. **6.8.3.** Verify all the steps are followed as per the Applikon Bioreactor Controller Operation SOP for preparing the bioreactor.	Operator/Date	Verifier/Date
6.8.4. Verify that the gas filters are open to avoid pressure difference during autoclaving.	Operator/Date	Verifier/Date

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6.8.5. Verify all tubing (near the head plate) except the condenser top outlet is clamped. The condenser top outlet must remain unclamped to release pressure during autoclaving.	Operator/Date	Verifier/Date
6.8.6. Cover the tubing and the head plate with aluminum foil. Place the autoclave indicator on the aluminum foil.	Operator/Date	Verifier/Date
6.9. Autoclave the bioreactor, alkaline addition bottle and liquid addition bottles as per section 8.2.6. Applikon Bioreactor Controller Operation SOP. Autoclave at 121°C for 20 minutes, using slow exhaust per Autoclave SOP. **CAUTION: Always use slow exhaust when autoclaving.**	Operator/Date	Verifier/Date
6.10 Remove the Bioreactor vessel, Alkaline Bottle, Media addition bottle, Inoculum addition bottle from the autoclave.	Operator/Date	Verifier/Date
7. Preparation of Cell Growth Media		
7.1.1. Obtain sterile two 500 mL bottles of DMEM Media. **DMEM Bottle 1:** Manufacturer:_____ Catalog number: _____ Lot number:_____ Expiration date:_____ **DMEM Bottle 2:** Manufacturer:_____ Catalog number: _____ Lot number:_____ Expiration date:_____	Operator/Date	Verifier/Date
7.1.2. Obtain sterile Superlow IgG Fetal Bovine Serum (FBS). Manufacturer:_____ Catalog number: _____ Lot number:_____ Expiration date:_____	Operator/Date	Verifier/Date
7.1.3. Obtain sterile 100X Insulin-Transferrin Selenium (ITS-G). Manufacturer:_____ Catalog number: _____ Lot number:_____ Expiration date:_____	Operator/Date	Verifier/Date
7.1.4.Obtain sterile 1000X (0.2mM) methotrexate solution. Manufacturer:_____ Catalog number: _____ Lot number:_____ Expiration date:_____	Operator/Date	Verifier/Date

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7.1.5. Obtain sterile 10 mg/mL gentamicin. Manufacturer:_____Catalog number: _____ Lot number:_____Expiration date:_____	Operator/Date	Verifier/Date
7.2. Place the autoclaved 1 L media addition in the Biological Safety Cabinet after swabbing it with 70% Ethanol. Do Not Remove the aluminum foil from the tubing and the filter.	Operator/Date	Verifier/Date
7.3. Place the media components 7.1.1, 7.1.2, 7.1.3, 7.1.4, 7.1.5 in the Biological Safety Cabinet after swabbing with 70% Ethanol.	Operator/Date	Verifier/Date
7.4.1. Aseptically remove 100 mL from one 500 mL bottle of pre-sterilized DMEM media and place in the empty two 50 ml conical tubes. **7.4.2**. Aseptically add the remaining 400 mL and an additional 500 mL bottle of pre-sterilized DMEM media to the 1L addition bottle with tubing and autoclavable male connector attached. **7.4.3.** Aseptically transfer the remaining media from each 500 mL bottle to the 1L addition bottle. **7.4.4.** Aseptically add 100 mL of Superlow IgG FBS to the media in the addition bottle. **7.4.5**. Aseptically add 10 mL of 100X ITS-G to the addition bottle. **7.4.6.** Aseptically add 1 mL of 1000X (0.2mM) methotrexate to the bottle. **7. 4.7.** Aseptically add 10 mL of 10 mg/mL gentamicin to the bottle.	Operator/Date	Verifier/Date
8. Connecting the bioreactor to the controller and preparing for the run. Refer to the steps 8.3.1 to 8.3.8 of the Applikon Bioreactor Controller Operation SOP.		
8.1 Verify that all control loops are switched off. Refer to the steps in section 8.3.1 of the Applikon ez-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.2. Transfer Cell Growth Media to Bioreactor. 8.2.1. Add 1L Cell Growth Media from 1L feed bottle to the bioreactor per steps 8.3.2.4. to 8.3.2.11 of the of the Applikon Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.3 Connect the sensors to the controller per step 8.3.3 of the Applikon ez-Control Bioreactor Controller Operation SOP.		

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8.4. Verify that deionized H_2O has been added to the thermowell with the Pt- 100 temperature probe. Add more deionized H_2O if necessary.	Operator/Date	Verifier/Date
8.5. Verify that thermal blanket is wrapped around the vessel and plugged into the ADI 1025 unit.	Operator/Date	Verifier/Date
8.6. Connect the stirrer motor by referring to the steps 8.3.7.1 to 8.3.7.5.of the Applikon Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.7. Connect the alkaline bottle. Refer to the steps 8.3.8.1 to 8.3.8.9 of the Applikon ez-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.8. Input the following limits per the process SOP. **Parameter** / **Upper limit** / **Set Point** / **Lower limit** pH: 7.3 / 7.2 / 7.1 Temperature: 38 / 37 / 36 Stirrer RPM: 76 / 75 / 74	Operator/Date	Verifier/Date

8.9. Turn on CO_2 supply at regulator to the bioreactor. Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
8.10 Start pH, Temperature and Stirrer control loop per step 8.3.10 of the Applikon ez-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
9. Media Hold and DO probe Polarization Perform Media hold and DO probe polarization simultaneously. Media should be held for 24 hrs. to check for contamination and DO probe should be polarized for atleast 6 hours before calibration.		
9.1 Media Hold Verify that all control loops are at set point.	Operator/Date	Verifier/Date
9.2. DO probe Polarization **9.2.1.** Connect DO probe to the controller.	Operator/Date	Verifier/Date

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9.2.2. Check media for contamination after a minimum of 24 hrs. Incubation start time:_____ Incubation end time: _____ Elapsed time: _____ Contamination? Yes / No (Circle one.)	Operator/Date	Verifier/Date
9.3. Start air compressor and set pressure at 10 psi.	Operator/Date	Verifier/Date
9.4. Calibrate the DO probe per the Applikon Bioreactor Controller Operation SOP (step 8.3.11-8.3.12). **Note:** Allow DO probe to polarize for at least 6 hours before performing calibration. Record slope: Slope _____ Temperature _____	Operator/Date	Verifier/Date
9.4.1 Set DO parameters as follows: Parameter — %DO Set point — 50 Upper Limit — 52 Lower Limit — 48	Operator/Date	Verifier/Date
9.4.2. Verify that slope is within expected values: 1.5-3.0 at 37°C or 2.0-4.0 at 25°C	Operator/Date	Verifier/Date
10. Inoculation of bioreactor with 100 ml spinner flask cell suspension Inoculate bioreactor when the cell suspension of CHO cells in the spinner reaches a concentration of ≥ 1x 10^6 cells/ml. Refer to step 8.4.1.1 to 8.4.1.11 of the Applikon ez-Control Bioreactor Controller Operation SOP.		
10.1 Obtain, clean autoclaved 500 mL feed bottle.	Operator/Date	Verifier/Date

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10.2. Aseptically transfer CHO DP12 cell suspension from spinner flask to 500 mL feed bottle per SOP. Volume of CHO DP12 suspension added _____	Operator/Date	Verifier/Date
10.3. Inoculate bioreactor with CHO DP12 cell suspension per steps 8.4.1 of the Applikon Bioreactor Controller SOP.	Operator/Date	Verifier/Date
Comments	Operator/Date	Verifier/Date
11. Monitoring of Bioreactor Cell Culture. Immediately after inoculation of the bioreactor (Day 0) and at 1 –day intervals, sample the culture to determine OD at 650 nm, viable cell count and viability, concentration of glucose, concentration of lactate, and anti-IL-8 concentration.	Operator/Date	Verifier/Date
11.1. Sampling Procedure Day 0- Day 2		

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11.1.1. Label 2 spectrophotometer cuvettes as “blank” and “sample”. 11.1.2. Label 5 microfuge tubes as follows: “cells”, “cell count”, “trypan blue” ,“balance”, “anti-IL-8-vessel name, day of culture, group initials date”. 11.1.3. Aseptically transfer 1 mL of blank solution from the tube labelled Blank to a microfuge tube labelled “Blank”. 11.1.4. Label 50 mL conical tube “anti- IL8, initial, date. 11.1.5. Log in to Applikon EZ Controller as operator per Applikon Operator SOP. 11.1.6. Raise the stirrer upper limit to 150 rpm. 11.1.7. Change the stirrer setting to 125 rpm. 11.1.8. Spray the head plate near the sampling tube with 70% IPA. 11.1.9. Remove the black clamp and set on the head plate. 11.1.10. Pull out the autoclavable female connector and set it next to the black clamp. 11.1.11. Place a 10 mL pipette into the sampling tube, remove 25 mL of sample and place sample in a 50 mL conical tube labelled sample. 11.1.12. Put the female autoclavable connector back into the sampling tube. 11.1.13. Bend the sampling tubing and place the black clamp back on the tubing. 11.1.14. Change the stirrer setting to 75 rpm. 11.1.15**.** Change the stirrer upper limit back to 76 rpm.	Operator/Date	Verifier/Date
Comments	Operator/Date	Verifier/Date
11.2. Testing Culture Samples- Day 0-Day2		

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11.2.2 OD 650nm Measurement 11.2.2.1 Remove 1 mL of sample from the 50 mL conical tube and place in spectrophotometer cuvette labeled "sample". 11.2.2.2. Remove 1 mL of blank from the microfuge tube and place into spectrophotometer cuvette labelled "Blank". 11.2.2.3. Measure OD at 650 nm and record the OD in the table on the page of the Batch Record. Return 1 mL sample back to the 50 ml conical tube labelled "sample".	Operator/Date	Verifier/Date
11.2.3. **Cell Concentration and Viability –Trypan Blue Assay** 11.2.3.1. Centrifuge the 50 mL tube containing sample at 900 rpm for 5 minutes. 11.2.3.2. Carefully remove supernatant, leaving approximately 0.2 mL behind so as not to disturb the pellet. Transfer supernatant to a new 50 mL conical tube. 11.2.3.3.Re-suspend the pellet in 0.8 mL of excess supernatant from step 8.6.2.2.2 using a 5 mL pipette. 11.2.3.4. Perform the trypan blue assay per SOP on the re-suspended pellet. Record the viable cell/ml and % viability in the table on the page the Batch Record.	Operator/Date	Verifier/Date
11.2.3. Anti-Il-8, Glucose, and Lactate Concentration 11.2.3.1. Remove 1mL of supernatant from 50 mL conical tube in step 8.6.2.2.2 and transfer to a microfuge tube labeled "anti-IL-8- bioreactor- time point- initials, date". 11.3.3..2. Store sample at 2-8°C in microfuge storage box labeled with date, group name, for measurement of glucose, lactate, and anti-IL8 concentration.	Operator/Date	Verifier/Date
11.3. Sampling Procedure- Day 3- End of Run (EOR)		
11.3.1. Label 15 mL conical tube "Anti-IL8, initial , date". 11.3.1. Label 2 spectrophotometer cuvettes as "blank" and "sample". 11.3.2. Label 5 microfuge tubes as follows: "cells", "cell count", "trypan blue" ,"balance", "anti-IL-8-vessel name, day of culture, group initials date". 11.3.3. In BSC, aseptically transfer 1 mL of blank solution from the tube labelled Blank to a microfuge tube labelled "Blank".	Operator/Date	Verifier/Date

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11.3.4. Log in to Applikon Bioreactor Controller operator. 11.3.5. Raise the stirrer upper limit to 150 rpm. 11.3.6. Change the stirrer setting to 125 rpm. 11.3.7. Spray the head plate near the sampling tube with 70% IPA. 11.3.8. Remove the black clamp and set on the head plate. 11.3.9. Pull out the autoclavable female connector and set it next to the black clamp. 11.3.10. Place a 10 mL pipette into the sampling tube, remove 5 mL of sample and place sample in a 15 mL conical tube labelled sample. 11.1.11. Put the female autoclavable connector back into the sampling tube. 11.1.12. Bend the sampling tubing and place the black clamp back on the tubing. 11.1.13. Change the stirrer setting to 75 rpm. 11.1.14**.** Change the stirrer upper limit back to 76 rpm. 11.3.15. Put the female autoclavable connector back into the sampling tube.	Operator/Date	Verifier/Date
11.4. Testing Culture Samples Day 3- End of Run (EOR)		
11.4.1 Cell Viability 11.4.1**.** Remove 100 µL from 15 mL conical tube containing 3 mL sample and place in microfuge tube labeled “cells count”. 11.4.2. Determine viable cell count per Trypan Blue Assay SOP. Record cell viability and concentration in the table of the Batch Record.	Operator/Date	Verifier/Date
11.4.2. OD 650 nm Measurement 11.4.2.1. Remove 1 mL of sample from the 15 mL conical tube containing 5 mL sample and place in spectrophotometer cuvette labeled “sample”. 11.4.2.2. Remove 1 mL of blank from the microfuge tube and place in spectrophotometer cuvette labelled “Blank”. 11.4.2.3. Measure OD at 650 nm. Record the OD in the table in the batch record.	Operator/Date	Verifier/Date

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11.4.3 Measurement of Glucose, Lactate, and Anti- IL-8 concentration 11.4.3.1 Remove 1 mL of the remaining 1.9 mL sample and place in the microfuge tube labelled "cells". Place 1 mL of millQ water in microfuge tube labeled "balance". Centrifuge both "cells" and "balance tubes" for 5 minutes in microcentrifuge. 11.4.3.2 Remove supernatant from the sample tube and transfer to microfuge tube labeled "anti- IL-8 vessel name (Spinner or Bioreactor), day of culture, group initials, date". 11.4.3.3. Store sample at 2-8°C in microfuge storage box labeled with date, group name, for measurement of glucose, lactate, and anti-IL8 concentration. 11.4.3.4 Add 10% bleach solution to the remaining sample and discard in the biohazard waste.	Operator/Date	Verifier/Date
12. Ending a Run	Operator/Date	Verifier/Date
12.1. Turn off each control loop refer to the Applikon ez-Control Bioreactor Controller Operation SOP 12.2. Turn off the supply of Air pump. 12.3. Turn off the supply of CO_2 tank.	Operator/Date	Verifier/Date
13. Harvest		
13.1. Refer to the SOP: Applikon ez-Control Bioreactor Controller Operation for instructions on removing the head plate of the bioreactor, providing access to the cells and conditioned medium. 13.2. Transfer the culture to three 250 mL centrifuge bottles using a 50 mL pipet and PipetAid. Residual culture can be transferred to an Ehrlenmeyer flask for temporary storage. 13.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 500 x g for 5 min, 4ºC. 13.4. Transfer conditioned medium (CM) from centrifuge bottle to storage bottle by carefully decanting the supernatant to appropriately labeled 250 mL Corning bottles.	Operator/Date	Verifier/Date
14. Clean pH and DO Probes		

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14.1. Clean the pH and DO probes with DI water. Spray with 70% IPA and pat dry with a lint-free laboratory wipe. 14.2. Store the pH probe in a pH storage solution in a storage bottle. 14.3. Store the DO probe in an electrolyte solution for short term. For long-term storage, store the DO probe dry. Replace the protective cap on the probe.	Operator/Date	Verifier/Date
15. Clean Bioreactor **Clean** the bioreactor per Applikon ez_Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
16. QC Biochemistry of the Samples from spinner and the Bioreactor.		
16.1. Perform the Quantitative Glucose Assay of all the spinner and Bioreactor samples per Glucose Determination Assay SOP.	Operator/Date	Verifier/Date
16.2. Perform the Quantitative Lactate Assay of all the spinner and Bioreactor samples per Lactate Determination Assay SOP.	Operator/Date	Verifier/Date
16.3 Perform the Anti-IL8 Mab Quantitative ELISA Assay of all the spinner and Bioreactor samples per Quantitation of CHO DP-12 derived Mouse anti-Human IL-8 Monoclonal Antibody by ELISA SOP.		
16.4. Prepare the growth curve for spinner flask samples and Bioreactor samples. **Spinner Flask** Cells/mL, glucose, and lactate vs. time (use 2 y-axes). Anti-IL-8 concentration and cells/mL vs. time (use 2 y-axes). Attach graphs to Batch Record. **Bioreactor** Cells/mL, % viability, and total cells vs. time (use 2 y-axes). Cells/mL, glucose, and lactate vs. time (use 2 y-axes). Anti-IL-8 concentration and cells/mL vs. time (use 2 y-axes). Attach graphs to Batch Record.	Operator/Date	Verifier/Date

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16.5 Attach QC data to the batch record.	Operator/Date	Verifier/Date
Comments	Operator/Date	Verifier/Date

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Lot Number ___________________________

APPLIKON BIOREACTOR ID # ____

TIME (hours)	OD 650nm	pH	Viable cells/mL	Percent Viability	GLUCOSE (mg/dl)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: Anti-IL-8 mAb Production from CHO DP-12 Cells
Lot Number ___________________________

APPLIKON BIOREACTOR ID # _______________________________________

TIME (hours)	OD 650nm	pH	Viable cells/mL	Percent Viability	GLUCOSE (mg/dl)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier