

Batch Record: HSA Production from *Pichia pastoris* Upstream Process
HSA 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. "If you didn't document it, you didn't do it!"

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: DDMMYY. For July 10, 2006 use 10JUL06.

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.

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1. Media Preparation for Seed Flask Cultures		
<p>Dissolve $1.3 \pm 0.05\text{g K}_2\text{HPO}_4$ and $5.8 \pm 0.05\text{g KH}_2\text{PO}_4$ into $500 \pm 5\text{mL}$ of deionized water in a 1L flask.</p> <p><u>K_2HPO_4 (potassium phosphate dibasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams</p> <p><u>KH_2PO_4 (potassium phosphate monobasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams</p> <p>Volume of water added: _____ mL</p>	Operator/Date	Verifier/Date
<p>Adjust 0.1M potassium phosphate buffer to pH 6 ± 0.1. pH _____</p>	Operator/Date	Verifier/Date
<p>Add $5 \pm 0.5\text{g}$ yeast extract to the potassium phosphate buffer.</p> <p>Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____</p> <p>Amount weighed: _____ grams</p>	Operator/Date	Verifier/Date
<p>Add $10 \pm 0.5\text{g}$ peptone to the potassium phosphate buffer.</p> <p>Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____</p> <p>Amount weighed: _____ grams</p>	Operator/Date	Verifier/Date
<p>Add $10 \pm 0.5\text{g}$ glucose to the potassium phosphate buffer.</p> <p>Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____</p> <p>Amount weighed: _____ grams</p>	Operator/Date	Verifier/Date

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<p>Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask.</p> <p>Shake Flask ID: _____</p> <p>Volume of media transferred _____ mL</p>	Operator/Date	Verifier/Date
<p>Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask.</p> <p>Shake Flask ID: _____</p> <p>Volume of media transferred: _____ mL</p>	Operator/Date	Verifier/Date
<p>Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask.</p> <p>Shake Flask ID: _____</p> <p>Volume of media transferred: _____ mL</p>	Operator/Date	Verifier/Date
<p>Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask.</p> <p>Shake Flask ID: _____</p> <p>Volume of media transferred: _____ mL</p>	Operator/Date	Verifier/Date
<p>Transfer 90mL of the 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone into a 500mL shake flask. for use in cryopreservation.</p> <p>Shake Flask ID: _____</p> <p>Volume of media transferred: _____ mL</p>	Operator/Date	Verifier/Date
<p>Transfer 36mL of the media into a 100mL bottle for blanking the spectrophotometer.</p> <p>Volume of media transferred _____ mL</p>	Operator/Date	Verifier/Date
<p>Autoclave 500mL flasks and 100mL bottle of media per autoclave SOP.</p> <p>Autoclave ID: _____</p>	Operator/Date	Verifier/Date

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<p>Prepare 10x YNB Solution: Weigh out 6.7±0.02g yeast nitrogen base without amino acids and combine with 100±1mL deionized water. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams Volume of water added: _____ mL</p>	Operator/Date	Verifier/Date
<p>Filter sterilize the 10X YNB and label as: Sterile Filtered 10X YNB, [date], [initials], Store: 2-8°C, Dispose: drain.</p>	Operator/Date	Verifier/Date
<p>Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _____ Group: _____ Volume of 10X YNB added to flask: _____</p>	Operator/Date	Verifier/Date
<p>Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _____ Group: _____ Volume of 10X YNB added to flask: _____</p>	Operator/Date	Verifier/Date
<p>Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _____ Group: _____ Volume of 10X YNB added to flask: _____</p>	Operator/Date	Verifier/Date
<p>Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media. Shake Flask I.D.: _____ Group: _____ Volume of 10X YNB added to flask: _____</p>	Operator/Date	Verifier/Date
<p>Aseptically add 10mL 10X YNB to the COOLED autoclaved flask of media containing 90mL of media for the purpose of cryopreservation. Shake Flask I.D.: _____ Volume of 10X YNB added to flask: _____</p>	Operator/Date	Verifier/Date

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<p>Aseptically add 4mL 10X YNB to the COOLED autoclaved 100mL glass bottle containing 36mL of media.</p> <p>Volume of 10X YNB added to 100mL bottle: _____</p>	Operator/Date	Verifier/Date
<p>Label the five shake flasks as: 0.1M Potassium Phosphate Media, pH 6, 1X YNB, with 1% Yeast Extract and 2% Peptone, [date], [group], [initials], Store: 2-8°C, Dispose: drain</p>	Operator/Date	Verifier/Date
<p>Label the 100mL bottle as: 0.1M Potassium Phosphate Media, pH 6, 1X YNB, with 1% Yeast Extract and 2% Peptone, [date], [initials], Blanking Media for Spectrophotometer, Store: 2-8°C, Dispose: drain.</p>	Operator/Date	Verifier/Date
<p>Proof the media in the shake flasks at 37 ± 0.5 °C shaking at approximately 200 rpm for a minimum of 24 hours.</p> <p>Incubation Time: _____</p>	Operator/Date	Verifier/Date
<p>Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group: _____ Contamination: YES/NO (Circle one) Bleached and disposed down drain: YES/NO (Circle one)</p>	Operator/Date	Verifier/Date
<p>Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group: _____ Contamination: YES/NO (Circle one)</p>	Operator/Date	Verifier/Date
<p>Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group: _____ Contamination: YES/NO (Circle one)</p>	Operator/Date	Verifier/Date
<p>Check media for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Group: _____ Contamination: YES/NO (Circle one)</p>	Operator/Date	Verifier/Date

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<p>Check media intended for cryopreservation for contamination. If contaminated, add bleach and dispose down drain. Shake Flask I.D.: _____ Contamination: YES/NO (Circle one)</p>	Operator/Date	Verifier/Date
<p>Comments:</p>	Operator/Date	Verifier/Date
<p>2. Seed Flask Culture</p>		
<p>Thaw contents of 1mL cryovials (one vial per shake media) of <i>Pichia pastoris</i> cells in 30°C water bath. Shake Flask ID _____ Vial ID _____ Shake Flask ID _____ Vial ID _____ Shake Flask ID _____ Vial ID _____ Shake Flask ID _____ Vial ID _____</p>	Operator/Date	Verifier/Date
<p>Prepare the biological safety cabinet (BSC) per the BSC SOP.</p>	Operator/Date	Verifier/Date
<p>Sterilely transfer the contents of each vial to a flask containing 100mL media. Label flasks as Pichia Inoculum, [group], [date], [initials]. Dispose: Autoclave then drain.</p>	Operator/Date	Verifier/Date
<p>Incubate flasks 24-48 hours in shaking incubator at 30°C at approx. 200 rpm.</p>	Operator/Date	Verifier/Date
<p>Aseptically remove a 2mL sample from each seed flask and place into a corresponding labeled cuvette. Take OD reading of cultures at 600nm. Shake Flask ID _____ Group _____ OD _____ Shake Flask ID _____ Group _____ OD _____ Shake Flask ID _____ Group _____ OD _____ Shake Flask ID _____ Group _____ OD _____</p>	Operator/Date	Verifier/Date

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<p>Prepare a Gram stain of each culture per the Gram Stain SOP. Examine for contamination of cultures.</p> <p>Shake Flask ID _____ Group _____ Contamination: YES/NO (Circle one)</p> <p>Shake Flask ID _____ Group _____ Contamination: YES/NO (Circle one)</p> <p>Shake Flask ID _____ Group _____ Contamination: YES/NO (Circle one)</p> <p>Shake Flask ID _____ Group _____ Contamination: YES/NO (Circle one)</p>	Operator/Date	Verifier/Date
<p>Comments:</p>	Operator/Date	Verifier/Date
<p>3. Media Preparation for Bioreactor</p>		
<p>Dissolve $2.3 \pm 0.05\text{g K}_2\text{HPO}_4$ and $10.4 \pm 0.05\text{g KH}_2\text{PO}_4$ in $900 \pm 10\text{mL}$ of deionized water in a 2L flask.</p> <p><u>K_2HPO_4 (potassium phosphate dibasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams</p> <p><u>KH_2PO_4 (potassium phosphate monobasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams</p> <p>Volume of water added: _____ mL</p>	Operator/Date	Verifier/Date
<p>Add 20 ± 0.5 grams glucose to the media.</p> <p>Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams</p>	Operator/Date	Verifier/Date
<p>Adjust 0.1M potassium phosphate buffer to pH 6 ± 0.1.</p> <p>pH _____</p>	Operator/Date	Verifier/Date

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Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Dissolve 2.3 ± 0.05g K ₂ HPO ₄ and 10.4 ± 0.05g KH ₂ PO ₄ into 900 ± 10mL of deionized water in a 2L flask. <u>K₂HPO₄ (potassium phosphate dibasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams <u>KH₂PO₄ (potassium phosphate monobasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams Volume of water added: _____ mL	Operator/Date	Verifier/Date
Add 20 ± 0.5 grams glucose to the media. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1. pH _____	Operator/Date	Verifier/Date
Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Dissolve 2.3 ± 0.05g K ₂ HPO ₄ and 10.4 ± 0.05g KH ₂ PO ₄ into 900 ± 10mL of deionized water in a 2L flask. <u>K₂HPO₄ (potassium phosphate dibasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams <u>KH₂PO₄ (potassium phosphate monobasic anhydrous)</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams Volume of water added: _____ mL	Operator/Date	Verifier/Date
Add 20 ± 0.5 grams glucose to the media. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date

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Adjust 0.1M potassium phosphate buffer to pH 6 ±0.1. pH _____	Operator/Date	Verifier/Date
Label flask as: 0.1M Potassium Phosphate Media, pH 6, [date], [initials], Store: 2-8°C, Dispose: drain.	Operator/Date	Verifier/Date
Prepare 300mL 10x YNB Solution: Weigh out 20.1±0.05g yeast nitrogen base without amino acids and combine with 300±5mL deionized water. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams Volume of water added: _____ mL	Operator/Date	Verifier/Date
Filter sterilize the 10X YNB and label as: Sterile Filtered 10X YNB, [date], [initials], Store: 2-8°C, Dispose: drain	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
4. Assemble BioFlo 3000 per BioFlo 3000 SOP		
Clean all bioreactor parts per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Assemble the vessel per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Assemble the headplate (underside) per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Aseptically add 2.7L of 0.1M Potassium Phosphate Media, pH 6 to the vessel per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Attach the headplate to the vessel per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Assemble the headplate (top side) per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Connect the bioreactor to the cabinet per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date

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<p>Calibrate the pH probe per the BioFlo 3000 SOP using commercially prepared standard buffers (pH 7 and pH 4): <u>pH 7 Buffer</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ <u>pH 4 Buffer</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____</p>	Operator/Date	Verifier/Date
<p>Apply a small amount of deionized water to the pH probe and then insert it into the pH probe port.</p>	Operator/Date	Verifier/Date
<p>Ensure that the pH probe is not touching the baffle.</p>	Operator/Date	Verifier/Date
<p>Install dissolved oxygen probe per BioFlo 3000 SOP.</p>	Operator/Date	Verifier/Date
<p>Remove protective cap from the bottom of the DO probe and inspect screen. Replace if damaged. Protective screen damaged? Yes / No (Circle one.) Protective screen replaced? Yes / No (Circle one.)</p>	Operator/Date	Verifier/Date
<p>Unscrew the bottom housing of the probe tip. 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.)</p>	Operator/Date	Verifier/Date
<p>Replenish DO electrolyte.</p>	Operator/Date	Verifier/Date
<p>Carefully insert the DO probe into the DO port of the headplate.</p>	Operator/Date	Verifier/Date
<p>Ensure that the DO probe is not touching the baffle.</p>	Operator/Date	Verifier/Date
<p>Attach tubing per BioFlo 3000 SOP.</p>	Operator/Date	Verifier/Date
<p>Autoclave the entire assembly at a minimum of 121°C for at least 30 minutes per BioFlo 3000 SOP and autoclave SOP.</p>	Operator/Date	Verifier/Date
<p>Aseptically add 300mL of filtered 10X YNB through the inoculation port.</p>	Operator/Date	Verifier/Date
<p>5. Prepare Feed Solutions for BioFlo 3000</p>		
<p>Assemble two 1L flasks (each with a sidearm) for feed solutions per process SOP.</p>	Operator/Date	Verifier/Date
<p>Autoclave the two assembled 1L flasks per autoclave SOP.</p>	Operator/Date	Verifier/Date

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Aseptically pour approximately 500mL of 30% NH ₄ OH into an assembled feed solution flask. CAUTION: Wear safety glasses and pour in a fume hood.	Operator/Date	Verifier/Date
Aseptically pour approximately 1L of 100% methanol into an assembled feed solution flask.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
6. Prepare Bioreactor for Operation		
Prepare the BioFlo 3000 for operation per the BioFlo 3000 SOP.	Operator/Date	Verifier/Date
When prompted by the BioFlo 3000 SOP, input the working temperature into the control panel of the bioreactor. Desired Working Temperature: 30°C	Operator/Date	Verifier/Date
Set up the 1L flask containing 30% NH ₄ OH solution on Feed 1 per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Set up the 1L flask containing 100% methanol solution on Feed 2 per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
When prompted by the BioFlo 3000 SOP, input the desired pH into the control panel of the bioreactor. Desired pH: 6.0	Operator/Date	Verifier/Date
Calibrate the dissolved oxygen probe per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Set DO mode to control by agitation only per BioFlo 3000 SOP.	Operator/Date	Verifier/Date
Set the minimum agitation rpm to 200.	Operator/Date	Verifier/Date
Set the maximum agitation rpm to 1000.	Operator/Date	Verifier/Date
Set the dissolved oxygen (DO) level to 30%.	Operator/Date	Verifier/Date

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Comments:	Operator/Date	Verifier/Date
7. Fermentation Procedure		
Set up and start the BioCommand Lite program according to the instructions in the Fermentation Procedure section of the BioFlo3000 SOP.	Operator/Date	Verifier/Date
Record Biocommand Lite File name:	Operator/Date	Verifier/Date
8. Inoculation Procedure		
Verify that the bioreactor has reached all of its setpoints and that the setpoint parameters are within range before inoculation.	Operator/Date	Verifier/Date
Choose the seed culture(s) that has the highest OD and has NO contamination to inoculate the BioFlo 3000. Record which shake flask(s) was used to inoculate bioreactor below: Shake Flask ID _____ OD _____ Group _____ Contamination: YES/NO (Circle one) Shake Flask ID _____ OD _____ Group _____ Contamination: YES/NO (Circle one) Shake Flask ID _____ OD _____ Group _____ Contamination: YES/NO (Circle one) Shake Flask ID _____ OD _____ Group _____ Contamination: YES/NO (Circle one)	Operator/Date	Verifier/Date
Aseptically inoculate the bioreactor per the process SOP.	Operator/Date	Verifier/Date
Immediately take a sample of the culture per the process SOP.	Operator/Date	Verifier/Date
Record all data obtained during sampling in the chart at the end of this batch record.	Operator/Date	Verifier/Date

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<p>When glucose levels reach an undetectable level move to stage 2 of growth (methanol feed).</p> <p>Elapsed Time when moved to stage 2: _____ hours</p> <p>OD when moved to stage 2: _____</p>	Operator/Date	Verifier/Date
<p>After 12-48 hours of methanol feed, harvest the culture.</p>	Operator/Date	Verifier/Date
<p>Comments:</p>	Operator/Date	Verifier/Date
<p>9. Data Collection and Cell Harvest</p>		
<p>Retrieve data generated by Biocommand Lite per BioFlo 3000 SOP.</p>	Operator/Date	Verifier/Date
<p>Using the sampling assembly, collect 1L of culture into sterile bottles through the harvest port.</p>	Operator/Date	Verifier/Date
<p>Transfer about 50mL of the culture into individual centrifuge tubes.</p>	Operator/Date	Verifier/Date
<p>Centrifuge at approximately 3000xg for 5-8 minutes. Remove supernatant and pour into sterile bottles by pouring into sterile bottles. Store at 2-8°C for use in Downstream Processing SOPs.</p>	Operator/Date	Verifier/Date
<p>Shut down and clean the BioFlo 3000 per BioFlo 3000 SOP.</p>	Operator/Date	Verifier/Date
<p>10. Clean the BioFlo 3000</p>		
<p>Clean the BioFlo 3000 per the BioFlo 3000 SOP.</p>	Operator/Date	Verifier/Date
<p>11. Cryopreservation</p>		
<p>Autoclave 50mL of 100% glycerol in a 100mL bottle per autoclave SOP.</p>	Operator/Date	Verifier/Date
<p>In the BSC, sterilely transfer about 50mL of the culture into individual centrifuge tubes.</p>	Operator/Date	Verifier/Date

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In the BSC pour off the supernatant into a waste container.	Operator/Date	Verifier/Date
Sterilely add 11mL of autoclaved glycerol to the 100mL of 0.1M Potassium Phosphate Media, pH 6, 1X YNB with 1% Yeast Extract and 2% Peptone set aside for cryopreservation in the process SOP to make the storage media.	Operator/Date	Verifier/Date
Aseptically add 5mL of the storage media to each centrifuge tube and resuspend the pelleted <i>Pichia</i> cells.	Operator/Date	Verifier/Date
Aseptically dispense 1mL aliquots to sterile 1.5mL cryovials. Label the cryovials: <i>P. pastoris</i> , HSA, [date], [initials], P[#]. Increase the passage number by one from the recorded Vial ID used in the seed flask culture.	Operator/Date	Verifier/Date
Place cryovials in a Styrofoam tube rack. Label container: <i>P. pastoris</i> , HSA, Working Cell Bank, [date], [initials], P[#]. Store at -86°C.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

