Montgomery County Community College
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Blue Bell, PA 19422

SOP: CHO DP12 Spinner Flask Harvest

Approvals:

Preparer: Robin Zuck — Date:25MAY2021
Reviewer: Hetal Doshi — Date: 27MAY2021
Reviewer: Dr. Maggie Bryans — Date: 02JUN2021

1. Purpose:

1.1. To harvest anti IL-8 mAb containing conditioned media from a Spinner Flask culture and prepare the harvested media for protein A chromatography using a gravity flow column.

2. Scope:

2.1. This SOP applies to the preparation of CHO DP12 conditioned media for the isolation of produced anti IL-8 monoclonal antibody using a gravity flow protein A column.

3. Summary of Method:

3.1. Transfer of the cell suspension from the spinner flask to 50ml centrifuge tubes.
3.2. Pellet the cells by centrifugation.
3.3. Clarify media by Microfiltration

4. References:

5. Responsibilities:

5.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
5.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

6. Equipment and Materials:

6.1. 100 ml Spinner flask culture cell 6 x10^5-1 x 10^6 cells/ml or 2 T75 flasks 80-100% confluent
6.2. 50 mL centrifuge tubes (2)
6.3. 0.2 micron vacuum sterile filteration unit with storage bottle, 100ml or 250ml bottle.
6.4. 25ml or 50ml serological pipettes and pipette aid
6.5.70% Ethanol solution
6.6. Laboratory wipes
6.7. 37˚C, 5% CO_2 Incubator
6.8. Biological Safety Cabinent
6.9. Benchtop Centrifuge

7. Procedure:

7.1. Transfer of culture from spinner flask to centrifuge tubes.
7.1.1. Remove the Spinner Flask or T75 flasks from the incubator and place in the BioSafety cabinent.

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7.1.2. Aseptically transfer the culture from the Spinner Flask or T75 flasks to one or two - 50 mL centrifuge tubes using 25ml or 50 mL serological pipets and a PipetAid.

7.2. Centrifuge the culture in a pre-chilled centrifuge and rotor at 1000 x *g* for 5 minutes, at 4 ˚ C.

7.3. Place the centrifuge tubes in the Biosafety cabinet after swabbing with 70% ethanol. Aseptically transfer the supernatant, the conditioned medium (CM), from the centrifuge tubes to a 100ml or 250ml 0.22µm sterile filter unit with storage bottle by carefully decanting the supernatant off the cell pellet or pipetting the supernatant using a 25ml or 50ml pipette.

7.4. Filter the supernatant by applying a vacuum. Remove the filter portion of the unit and cap the bottle. Label the bottle "Anti-IL8 mAb conditioned media ,[Date], [final cell concentration],[Initial], [volume].Store the Conditioned Media at 4˚C for further processing

9. Attachments:

10. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0		Robin Zuck	Initial release