Application Protocol

Protocol for MRC-5 Cultivation in Thermo Scientific Nunc High Density Cell Factory Systems

Introduction:

This protocol was developed to validate the performance of the Thermo Scientific™ Nunc™ High Density Cell Factory™ System (HDCF) for the culturing of MRC-5 cells. The protocol includes reference to the use of standard Nunc Cell Factory systems as controls. It is a recommendation. This protocol may be used as a reference or offer options for optimizing the performance of established protocols.

Thawing of cells

1. Thaw 1 vial of MRC-5 cells with approximately 2.6 x 10^6 cells in 37°C water bath - thaw until there is a minimal amount of ice remaining.
2. Decontaminate the exterior of the vial with 70% ethanol or a similar decontamination solution.
3. Transfer the cell suspension from the vial to a 15mL centrifuge tube containing 9mL recommended growth media.
4. Recommended growth media:

Media Composition
Thermo Scientific™ Gibco™ DMEM, high glucose, with NEAA, no glutamine, with phenol red (liquid)
Gibco 100U/mL Penicillin + 100µg/mL Streptomycin (Stock solution: 10,000U/mL Penicillin + 10,000µg/mL Streptomycin)
Gibco 2mM L-Glutamine (Stock solution: L-Glutamine 200mM (100x))
Gibco 10% Fetal Bovine Serum, Certified, USA
Gibco 5-10mM HEPES (pH=7.2) (Stock solution: 1M HEPES (pH=7.2))(optional)

Recommended reagents:

Reagents
Gibco Dulbecco's Phosphate Buffered Saline solution without Ca^{2+} and Mg^{2+} (Liquid)
Gibco 0.25% Trypsin/EDTA

5. Gently triturate the cell suspension with a pipette.

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Passage 1:

1. Seed a T-175 flask containing 50mL recommended growth media with 15,000 cells/cm^2.
2. Incubate the T-175 flask with the cells for 7 days at 37°C under 5% CO_2 aeration.

Please note: If CO_2 aeration is not available, add HEPES to the culture media and incubate at 37 °C.

3. Take a sample from each unit for measurement of glucose, lactate and other metabolites, e.g. pH, glutamate and/or ammonium.
4. Remove the remaining media from the cells.
5. Wash with 10mL DPBS with no Ca^{2+} and Mg^{2+} per layer.
6. Add 5mL 0.25% Trypsin-EDTA.
7. Incubate for 2-3 minutes or until cell layer detachment can be verified visually.
8. Inactivate the Trypsin-EDTA with 20mL growth media and collect the cell suspension.
9. Gently triturate the cell suspension with a pipette.
10. Sample a small amount of the cell suspension for cell counting.
11. Count the cells using available methods and record the counts/cell concentrations for both viable and non-viable cells.
12. Use the cell count to determine the amount of cell suspension needed, to reach the intended cell density of the next vessel.

Passage 2: Cell Expansion in T-Flasks

1. Plate 6 x T-175 flasks with 15,000 cells/cm^2 using 50mL of recommended growth media for each T-flask.
2. Incubate the T175 flasks for 7 days at 37°C with 5% CO_2 aeration.

Please note: If CO_2 aeration is not available, add HEPES to growth media and incubate at 37°C.

3. Repeat steps 3-12 from Passage 1 for each T-175 flask.

Passage 3-5: Cell Expansion in HDCF systems

1. Plate 2x CF2 (control) and 2x HDCF3 with 15,000 cells/cm^2 using 200mL per layer of recommended growth media.

Cell Factory System	Media volume per system (200mL per layer)
CF2	400mL
HDCF3	600mL

2. Incubate the CF and HDCF units for 6 days at 37°C.
3. Take a sample from each unit for measurement of glucose, lactate and other metabolites, e.g. pH, glutamate and/or ammonium.
4. Remove the remaining media from the cells.
5. Wash with 40mL per layer DPBS with no Ca^{2+} and Mg^{2+} per layer.

Cell Factory System	DPBS volume per system (40mL per layer)
CF2	80mL
HDCF3	120mL

6. Discard the used wash buffer.
7. Add 15mL per layer 0.25% trypsin/EDTA per layer.

Cell Factory System	Trypsin-EDTA volume per system (15mL per layer)
CF2	30mL
HDCF3	45mL

8. Incubate for 4-5 minutes or until cell detachment is visually verified.

9 Inactivate the trypsin/EDTA with 40mL per layer recommended growth media.

Cell Factory System	Growth media volume per system (40mL per layer)
CF2	80mL
HDCF3	120mL

10. Collect the cell suspension in a suitable sterile collection vessel, e.g. single-use bottle or carboy.
11. Repeat step 9 to remove as many cells are removed from the CF/HDCF systems as possible.
12. Collect and pool the remaining cell suspension.
13. Agitate the collection vessel by gentle swirling and/or rotation, ensuring a homogenous cell suspension.
14. Sample a small volume of cell suspension from the middle of each collection vessel.
15. Count the cells using available methods and record the count.
16. Use the cell count to determine the amount of cell suspension needed, to reach the intended cell density of the next vessel.

Passaging into HDCF13 systems

1. Plate 3x CF10 (control) and 3x HDCF13 with a cell concentration of 15,000 cells/cm^2, and with 200mL per layer recommended growth media.

Cell Factory System	Media volume per system (200mL per layer)
CF10	2L
HDCF13	2.6L

2. Incubate for 6 days in a 37°C heated space.
3. Take a sample from each unit for measurement of glucose, lactate and other metabolites, e.g. pH, glutamate and/or ammonium.
4. Remove the remaining media from the cells.
5. Wash with 40mL per layer DPBS with no Ca^{2+} and Mg^{2+} per layer.

Cell Factory System	DPBS volume per system (40mL per layer)
CF10	400mL
HDCF13	520mL

6. Discard the used wash buffer.
7. Add 15mL per layer 0.25% trypsin/EDTA.

Cell Factory System	Trypsin-EDTA volume per system (15mL per layer)
CF10	150mL
HDCF13	195mL

8. Incubate for 4-5 minutes or until detachment is visually verified.
9. Inactivate the trypsin/EDTA with 40mL per layer recommended growth media per layer.

Cell Factory System	Growth media volume per system (40mL per layer)
CF10	400mL
HDCF13	520mL

10. Collect the cell suspension in a suitable sterile collection vessel.
11. Repeat step 9 to ensure complete removal of cell from the CF/HDCF systems.
12. Collect and pool the remaining cell suspension into the collection vessel.
13. Count the cells using available methods and record the count.

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