Protocol for Vero cultivation

Nunc High Density Cell Factory systems

Introduction

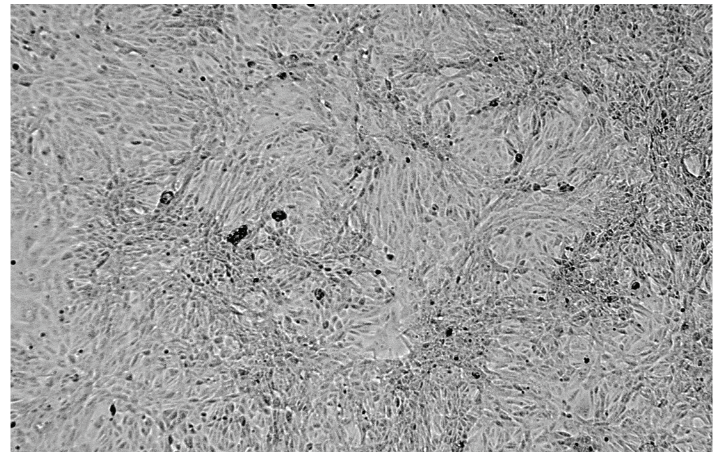
This protocol was developed to validate the performance of Thermo Scientific™ Nunc™ High Density Cell Factory™ systems for the culturing of Vero cells. The protocol includes the recommended use of standard Nunc Cell Factory systems as controls. This protocol may be used as a reference or as a resource for options to optimize the performance of established protocols.

Cell thawing

1. Thaw 1 vial of Vero cells with approximately 2 x 10^6 cells in a 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 15 mL centrifuge tube containing 9 mL of the recommended growth medium.
   - Recommended growth medium:
     – Gibco™ DMEM, high glucose, with NEAA, no glutamine, with phenol red (liquid)
     – 100 U/mL penicillin, 100 µg/mL streptomycin (stock solution: Gibco™ Penicillin-Streptomycin, 10,000 U/mL penicillin + 10,000 µg/mL streptomycin (100X))
     – 2 mM L-glutamine (stock solution: Gibco™ 200 mM L-glutamine (100X))
     – 10% FBS (Gibco™ Fetal Bovine Serum, Certified, US origin)
     – 5–10 mM HEPES (pH 7.2) (stock solution: Gibco™ 1 M HEPES)
     [deliberately omitted pH 7.2 from the HEPES stock solution]



   - Recommended reagents:
     – Gibco™ DPBS without Ca^{2+} and Mg^{2+}
     – Gibco™ 0.25% Trypsin-EDTA

4. Gently triturate the cell suspension with a pipette.

Passage 1

1. Seed a T-175 flask containing 50 mL of the recommended growth medium with 15,000 cells/cm^2.
2. Incubate the T-175 flask with the cells for 6 days at 37°C under 5% CO_2 aeration. Please note: If CO_2 aeration is not available, add HEPES up to a final concentration of 10 mM to the culture medium and incubate in a 37°C heated space.
3. Take a sample from each T-Flask for measurement of glucose, lactate, and other metabolites (e.g., pH, glutamate, and ammonium).
4. Remove the remaining medium from the cells.
5. Wash with 10 mL DPBS with no Ca^{2+} and Mg^{2+}.
6. Add 5 mL 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 20 mL growth medium 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 for both viable and nonviable 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 3 x T-175 flasks with 5,000 cells/cm^2 using 50 mL of the recommended growth medium for each T-flask.
2. Incubate the T-175 flasks for 4 days at 37°C with 5% CO_2 aeration. Please note: If CO_2 aeration is not available, add HEPES to growth medium and incubate at 37°C.
3. Repeat steps 3–12 from passage 1 for each T-175 flask.

Passage 3–5: cell expansion in High Density Cell Factory systems

1. Plate 1 x Cell Factory 2-layer system (control) and 1 x Cell Factory 2-layer system with 5,000 cells/cm^2 using 200 mL per layer of the recommended growth medium.

Cell Factory system	Medium volume per system
Cell Factory 2-layer system	400 mL (200 mL per layer)
High Density Cell Factory 3-layer system	600 mL (200 mL per layer)

2. Incubate the Cell Factory systems for 7 days at 37°C.
3. Take a sample from each unit for measurement of glucose, lactate, and other metabolites (e.g., pH, glutamate, and ammonium).
4. Remove the remaining medium from the cells.
5. Wash with 40 mL DPBS with no Ca^{2+} and Mg^{2+} per layer.

Cell Factory system	DPBS volume per system
Cell Factory 2-layer system	80 mL (40 mL per layer)
High Density Cell Factory 3-layer system	120 mL (40 mL per layer)

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

Cell Factory system	Trypsin-EDTA volume per system
Cell Factory 2-layer system	30 mL (15 mL per layer)
High Density Cell Factory 3-layer system	45 mL (15 mL per layer)

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

Cell Factory system	Growth medium volume per system
Cell Factory 2-layer system	80 mL (40 mL per layer)
High Density Cell Factory 3-layer system	120 mL (40 mL per layer)

10. Collect the cell suspension in a suitable sterile collection vessel (e.g., single-use bottle or carboy).

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11. Repeat step 9 to remove as many cells from the Cell Factory systems as possible.

12. Collect and pool the remaining cell suspension.

13. Agitate the collection vessel by gentle swirling or rotation, ensuring a homogeneous 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 in the next vessel.

Passaging into High Density Cell Factory 13-layer systems

1. Plate 3 x Cell Factory 10-layer system (control) and 3 x High Density Cell Factory 13-layer system with 15,000 cells/cm^2, and with 200 mL per layer recommended growth medium.

Cell Factory system	Medium volume per system
Cell Factory 2-layer system	2 L (200 mL per layer)
High Density Cell Factory 3-layer system	2.6 L (200 mL per layer)

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 ammonium).

4. Remove the remaining medium from the cells.

5. Wash with 40 mL DPBS with no Ca^{2+} and Mg^{2+} per layer.

Cell Factory system	DPBS volume per system
Cell Factory 10-layer system	400 mL (40 mL per layer)
High Density Cell Factory 13-layer system	520 mL (40 mL per layer)

6. Discard the used wash buffer.

7. Add 15 mL 0.25% trypsin-EDTA per layer.

Cell Factory system	Trypsin-EDTA volume per system
Cell Factory 10-layer system	150 mL (15 mL per layer)
High Density Cell Factory 13-layer system	195 mL (15 mL per layer)

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

9. Inactivate the trypsin-EDTA with 40 mL of the recommended growth medium per layer.

Cell Factory system	Growth medium volume per system
Cell Factory 10-layer system	400 mL (40 mL per layer)
High Density Cell Factory 13-layer system	520 mL (40 mL per layer)

10. Collect the cell suspension in a suitable sterile collection vessel.

11. Repeat step 9 to ensure complete removal of cells from the Cell Factory systems.

12. Collect and pool the remaining cell suspension into the collection vessel.

13. Count the cells using available methods and record the count.

Find out more at **thermofisher.com/cellfactory**

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