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Long term cell culture of HeLa cells: Impact of Medium pH

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We use this protocol and it's working

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Disclaimer

The content of this article follows the recommendations of Official Mexican Standard NOM-052-SEMARNAT-2005, which establishes the characteristics, identification procedure, classification, and lists of hazardous waste. Mention of trade names or commercial products does not constitute endorsement or recommendations for use.

Abstract

This protocol describes long term cell culture of HeLa cells with impact of medium pH. We present a culture method capable of stably maintaining an acidic pH over extended periods in HeLa cells, providing a reproducible platform to study chronic acidosis and to evaluate pH-dependent therapies.

Guidelines

Researchers should have completed basic laboratory safety, chemical safety, bloodborne pathogens, and biosafety level 2 (BSL-2), training prior to conducting this protocol.

Materials

Reagents

- Dulbecco Modified Eagle Medium (DMEM, Thermo Fisher)
- Fetal Bovine Serum (FBS, Thermo Fisher)
- Penicillin-Streptomycin (Thermo Fisher)
- Trypsin (Gibco #25200-056)

Equipment/Supplies

- Biosafety cabinet
- Humidified tissue culture incubator with 5% CO_2
- Tissue culture dishes
- Vacuum-driven bottle filter with 0.2 µm pore
- Pipetaid
- 50mL conical tubes
- Tabletop centrifuge
- Pipettes
- Hemocytometer

Safety warnings

None.

Prerequisites

1 **Reagents**
- Dulbecco Modified Eagle Medium (DMEM, Thermo Fisher)
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Objective

4 Development of a culture method in the HeLa cell line for the evaluation of pH-dependent therapies.

Factors and levels

5 pH = [7.4, 7.0, 6.8]
Adaptation = [chronic ≥10 passages]
Seeding density 1×10^6^

Units of analysis

6 Independent culture

Controls

7 Neutral pH (7.4)

Buffer only (PBS 1X)

Inclusion/exclusion criteria

8 pH out of range
Contamination

Procedure

8.1 Dissolve 4.75g of DMEM powder without phenol red, without L-glutamine in 500mL of distilled water.

8.2 Prepare a sterile solution of 0.33M NaHCO3. Sterilize by filtration.

8.3 Sterilize the medium in an autoclave (15 min, 0.1-0.11 MPa, 212°C [liquid cycle]). Allow to cool to 25°C.

8.4 Add 0.5g glucose, 5mL penicillin-streptomycin, and 50mL fetal bovine serum to the bicarbonate-free DMEM.

8.5 For the control medium (pH 7.4), add 2.4mL of 0.33M NaHCO3 solution for every 100mL of bicarbonate-free DMEM.

8.6 Check the pH of the medium after 24 hours of incubation at 37°C with 5% CO_2.
Note: The amount of NaHCO3 solution must be adjusted according to the pH.
Commercial products that can be used in this study are listed in the Materials table.

8.7 Add 150µL of 0.1M HCl to 100mL of control medium.

8.8 Check the pH of the medium after 24 hours of incubation at 37°C with 5% CO_2.
Note: The amount of HCl should also be adjusted according to the required pH.

8.9 Start culturing 1×10^6^ HeLa cells with control medium at 37°C with 5% CO_2 at least one week before starting treatment with acidic DMEM.

8.10 Change the control medium every 48 hours.

8.11 Pass the cells when they reach 70-80% confluence.

8.12 Wash the cells by adding 5mL of sterile 1X PBS.

8.13 Aspirate the PBS and add a detachable enzyme (TrypLE™ Express [1X]), 3mL.

8.14 Incubate at 37°C for 6 min. You should notice the cells rounding up and beginning to detach.

8.15 Add 3 mL of control DMEM culture medium to the detached cells to deactivate the enzyme.

8.16 Transfer the cell suspension to a 15 mL tube and centrifuge for 6 min at 1500 rpm.

8.17 Aspirate the supernatant and resuspend the cells with 5 mL of control DMEM culture medium.

8.18 Count viable cells using a Neubauer chamber and trypan blue.

8.19 Begin culturing HeLa cells from the standard culture.

8.20 Transfer (1×10^6^) HeLa cells to T25 flasks.

8.21 The final volume of the flask will be 5 mL of DMEM control (pH 7.4).

8.22 After 3 hours, verify that the cells begin to adhere to the substrate using a bright field microscope.

8.23 Aspirate the control medium and add 5 mL of medium at a lower pH.
Note: It is recommended to start the extracellular acidity treatment at pH 7.2 and renew the acidic medium every 48 hours.

Quality Control Rationale

9 Cells will be visually observed for culture quality prior to every passage.

Protocol references

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