Product Name:	**QuaCell® LeBit04 Medium**
Cat. No.	**A52002**
Amount:	**10L, 100L**
Formulation:	**Powder**
Storage:	**2°C ~8°C**
Validity period:	**24 months (Validity period on product packaging)**

Description

QuaCell® LeBit04 Medium is a chemically defined medium optimized for adeno-associated virus (AAV), lentivirus (LV) production in a variety of human embryonic kidney HEK 293 cell lines. QuaCell® LeBit04 Medium was streamlined and cell medium components were added to improve medium stability and reduce protein oxidation. The medium is animal-free and chemically defined.

Components

L-glutamine	No
Glucose	6.5 g/L
Hypoxanthine & Thymidine	Yes
Phenol red	No
Sodium bicarbonate	No
Hydrolysate	No

Product Intended Use

Use aseptic technique when handling or supplementing this medium. This product is for research or for further manufacturing use.

Caution: Not for human or animal therapeutic use. Uses other than the intended use may be a violation of local law.

Safety information

Read the Material Safety Data Sheets (MSDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparation Instructions

• Preparation method

1. Add 80% of the final volume of water for injection to a suitable clean container and adjust the water temperature to 20℃~ 30℃;

2. Slowly add 19.20 g /L QuaCell® LeBit04 Medium powder and stir until no powder clumps remain for about 15 minutes. In this step, the solution will not be clear, but no clumps will be present;

3. Slowly add 5 mol/L NaOH adjust the pH to 8.90±0.1 and stir 30 minutes;

4. Slowly add 2.5 g/L $NaHCO_3$ and stir 10 minutes until dissolved;

5. Add 5mol/L HCl to adjust the pH to 7.2±0.2, stir 10 minutes;

6. Add water for injection to the desired final volume, stir 10 minutes and mix well;

7. Sterilize by filtration using pore size 0.20μm filter, In 2℃~ 8℃ avoid light preservation.

• QuaCell® LeBit04 Medium under sterile condition.

• The product does not contain L-glutamine, Add L-glutamine as needed before use.The recommended final concentration is 6 mM. Prepare a 200 mM stock solution and add it just before use;

• The unused media after opening should be sub package, Use sealing film to seal, In 2℃~ 8℃ avoid light preservation.

Condition of cell culture

Medium: QuaCell® LeBit04 Medium

Cell Line: HEK293 cells

Culture type: suspension

Culture container: shaker /TPP/ reactor

Temperature range: 37℃±0.5℃

Incubator air requirements: humidified with 5%~8% CO_2

Note: ensure proper air exchange and minimum exposure.

Cell recovery

1. Quickly thawing cryopreservation tube in 37℃water, process about 1~2 minutes;

2.Add glutamine as needed before use according to the characteristics of cell lines;

3.Transfer cell liquid to 5 mL of preheating QuaCell® LeBit04 Medium, centrifugate at 1,000 rpm for 5 min, discard the supernatant, using 5 mL QuaCell® LeBit04 Medium suspension;

4. Use an automated cell counter or other counting instrument to count cells, pipette the cell fluid to a 125 mL shaker flask as needed for cell density, and add an appropriate amount of QuaCell® LeBit04 Medium to achieve the desired resuscitation density; the recommended resuscitation density is $(3\sim5)\times10^5$ cells/mL.

4. Culture in incubator contains 5% ~ 8% CO_2, 37℃ humidified air.

5. Cells should be cultured for 2 to 5 days after cell recovery and passed on in the middle stage of logarithmic growth. The recovered cells should be passed on at least three times before other experiments.

Cell passage advice

1. Use automatic cell counting apparatus or other counting instruments to count the cells, and passage them according to the required density or in proportion. Recommended inoculation density is $(3\sim5)\times10^5$ cells/mL.

2. Add the cells to a shaker flask containing QuaCell® LeBit04 Medium to achieve the desired seeding density of the cells.

3. Continue culture at 37℃, 5% ~ 8% CO_2 in the shake incubator, usually passage cells after 2 ~ 3 days.

Adaptation

Some HEK293 suspension cultures can be directly cultured into QuaCell® LeBit04 Medium from serum-supplemented or serum-

free medium with little or no adaptation.
It is essential that the cell is in the middle of logarithmic growth and make sure the cell viability is beyond 90% before the initiation of the adaption process.

Directly culture
Transfer the suspension culture cells to QuaCell® LeBit04 Medium, as follows:
1. Centrifuge cell suspension at 1000rpm for 5 minutes, remove and discard the supernatant.
2. Suspension cells into preheat fully QuaCell® LeBit04 Medium in (3 ~ 5) X 10^5 cells/mL of living cells density and transfer to an appropriate culture flask.
3. Put culture flask back to the shake incubator and observe cell growth.
Note: if unsatisfactory cell growth is observed using procedure adaption as follows.

Procedure adaption
Steps of cell suspension culture:
1. Make cell density of (4 ~ 5) X 10^5 cells/mL during the adaptation process.
2. Gradual adjustment QuaCell® LeBit04 Medium with the original proportion of cell culture medium (25:75, 50:50, 75:25, 90:10, then 100% QuaCell® LeBit04 Medium). Cells can be passaged several times depending on the situation in each step.
3. After been passaged in 100% QuaCell® LeBit04 Medium a few times, the living cell density should beyond (1~2) X 10^6 cells/mL, The cell viability within 4 ~ 6 days was greater than or equal to 85%. In this phase, cells are considered suitable for Wayne293® Transfection Medium. In the final stage of adaptation, the cell density can be reduced to (2~3) X 10^5 cells/mL.

Cryopreservation
1. Prepare the required number of cells and the cells are in good condition.
2. Use an automatic cell counter or other counting instrument for cell counting, and calculate the volume required for cryopreservation medium according to the final cryopreservation density, and the recommended cryopreservation density is >1×10^7 cells/mL.
3. Centrifugate at 1,000 rpm for 5 min, discard the supernatant, and resuspend the cells with an appropriate amount of cryopreservation medium to cryopreservation density.
4. The cell suspension should be immediately repackaged into a cryopreservation tube according to the specifications
5. According to the standard procedure, cryopreservation in automatic or manual control freezing equipment. Transfer cells into the liquid nitrogen, stored under -130℃.
Note: after 24 hours of storage in liquid nitrogen, remove one to examine the viability and other indicators. See "cell recovery".

Feeding advice
Different strategies for adding feed medium are recommended according to different uses.
- Feeding strategies for transient AAV/LV in 293 cell lines:

1. Enhancer and feed medium were added 24h after transfection, and feed medium (3% CellBest 007A Feed and 0.3% CellBest 007B Feed) was added every other day.
2. Test the glucose content of the system daily after feeding, and supplement less than 2 g/L to 4 g/L.
3. Cultures were stopped at < 50% viability to measure expression and other data for analysis.

• If the project already has a mature culture process, it is recommended to use the original process for trial. If the process is in development stage, it is recommended to use the DOE method to determine the appropriate culture parameters and obtain better results.

Related Products

Cat.No.	Product
A12907	QuaCell® CellBest 007 Feed Medium

Explanation of Symbols and Warnings

STERILE A		
Sterilized using aseptic processing techniques	Use By:	Store Temperature
LOT		
Batch code	Dry preservation	Keep away from light
RUO	GMP	
Research use only	GMP Manufacturing	Sticky notes