Cellplus Bio

MetaCell® TransAAV 01

User Manual

Product Description

MetaCell® TransAAV 01 is a culture medium designed to meet the packaging needs for various AAV (Adeno-Associated Virus) serotypes using multiple HEK293 cell lines. This medium is free of hydrolysates, proteins, and any animal-derived components, supporting high-density growth and transient transfection of various HEK293 cells, especially VPC2.0 and 293F cells. The components of the medium are highly optimized and compatible with commercial cationic transfection reagents.

MetaCell® TransAAV 01 contains 4mM glutamine derivative.

Product Name	Cat No.	Size	Storage	Shelf Life	Application
MetaCell® TransAAV 01	L2010-0500	500mL	2-8°C, protected from light	12 months	Efficient transient transfection of HEK293 cells (protein or viral expression)
	L2010-1000	1000mL			

This product is intended for research or further manufacturing but not for human or therapeutic use.

Cell Culture Conditions

Medium: : MetaCell® TransAAV 01

Application: Suspension cell culture

Cell line: Expi293F™, FreeStyle™ 293-F, 293F, VPC2.0

Recommended parameters for trials:

Shake flask volume	125mL	250mL	500mL	1L	3L	5L
Medium volume	30-35mL	60-70mL	120-140mL	240-280mL	600-1000mL	1500-2000mL
Shaker speed	125±5 rpm (amplitude19mm) 120± 5 rpm (amplitude25mm) 95±5 rpm (amplitude50mm)				105±5 rpm 95±5 rpm 80±5 rpm	
Types of flasks	PETG or PC, breathable, without baffles					
Culture environment	37 ± 0.5 ℃, 8% CO_2, humidity ≥80%, Ensure proper gas exchange and minimize light exposure during cultivation					

Cell Recovery

1. Cells transported on dry ice should be placed in a liquid nitrogen environment for 3-7 days before cell recovery.
2. Take 39mL of MetaCell® TransAAV 01 in advance and preheat it at 37 ℃ in a 125mL shake flask.
3. Remove a vial of frozen cells from the liquid nitrogen tank and thaw in a 37°C water bath (1-2 minute).
4. Transfer the cells to a centrifuge tube containing 9 mL of pre-heated MetaCell® TransAAV 01.
5. Centrifuge at 1000rpm for 4 minutes, discard the supernatant, resuspend the cells in pre-heated MetaCell®

TransAAV 01, and transfer all to a 125mL shake flask to make a final volume of 30mL.

6. After 3-4 days of cultivation, subsequent experiments should be carried out when the cell density is ≥ 3.0 x 10^6 cells/mL and the viability is ≥ 90%.
7. We recommend to passage the culture for at least three passages before starting subsequent experiments.

Cell Passaging

1. Pre-heat MetaCell® TransAAV 01 at 37°C for 20-30 minutes.
2. When the cell density reaches 3.0-4.0×10^6 cells/mL and the cell viability is ≥95% （day2-day4）, passaging culture can be performed.

Note: Different types of HEK293 cells may have different ranges of logarithmic growth phases, and the passaging time needs to be determined according to the actual situation to ensure that passaging culture is carried out in the early logarithmic growth phase.

3. The recommended cell seeding density is 0.4-0.6×10^6 cells/mL.
4. Transfer the required amount of seed solution to the shake flask, add an appropriate amount of pre-heated medium, set the parameters of the shaker according to the culture conditions, and passage the cells every 2-4 days using fresh medium following the above steps.
5. Cells should be passaged at least three times after thawing and recovery before subsequent experiments.

Cell Cryopreservation

1. Prepare a sufficient number of cells in the early logarithmic growth phase with a cell viability ≥95% for cryopreservation.
2. The final cell concentration for cryopreservation should be controlled at 10.0-15.0×10^6 cells/mL.
3. Cryopreservation solution: (90% MetaCell® TransAAV 01 + 10% DMSO), precool at 2-8°C for at least 30 minutes.
4. Take an appropriate amount of cell suspension, centrifuge at 1000rpm for 4 minutes, discard the supernatant, and resuspend the cells in the pre-cooled cryopreservation solution.
5. Divide the cell suspension into cryotubes according to the cryopreservation specifications.
6. Gradually cool the cells to -80°C for freezing (cooling rate of 1°C/min) using a controlled-rate freezer or manual control method.
7. After 24 hours, transfer the frozen cells to the vapor phase of a liquid nitrogen tank (storage temperature range: -200°C to -125°C) for storage.

Cell Adaptation

In most cases, serum-free cultured HEK293 cells can be directly adapted to MetaCell® TransAAV 01. If direct replacement of the medium (direct adaptation) fails, it is recommended to use gradient replacement (indirect adaptation) to adapt HEK293 cells to MetaCell® TransAAV 01.

Note: HEK-293 cells used for adaptation need to be in the early logarithmic growth phase, with a cell viability ≥95%.

• Direct Adaptation Method

For cells that can be directly adapted, when the cell viability is ≥95% and in the early logarithmic growth phase, try

directly transferring from serum-free medium to MetaCell® TransAAV 01.

1. Inoculate HEK293 cells into fresh MetaCell® TransAAV 01 at a seeding density of 0.4-0.6×10^6 cells/mL (refer to the cell passaging steps).
2. After 3-4 days of culture, check the cell density and viability. At this time, the cell viability should be ≥95%. If the viability is low, replace the adapted cells or use the indirect adaptation method.
3. Continue passage 3-4 times. When the cell density reaches 3.0-4.0 x 10^6 cells/mL, and cell viability is ≥95%, it can be considered that the cells have been adapted.

• Indirect Adaptation Method

1. Mix the original medium and MetaCell® TransAAV 01 at a volume ratio of 75:25, and the cell seeding density should be 0.4-0.6×10^6 cells/mL.
2. Cells should be passaged when the cell density reaches 3.0-4.0×10^6 cells/mL.
   (1) If the cells grow well and the viability is >90%, adjust the ratio of MetaCell® TransAAV 01 to the original medium to 50:50 during passaging.
   (2) If the cells grow slowly, cells can be subjected to centrifugation and media exchange, with centrifugation conditions at 1000rpm for 4 minutes. The mixed medium at this point still consists of MetaCell® TransAAV 01 and the original medium at a ratio of 25:75.
3. Repeat step 2, gradually increasing the proportion of MetaCell® TransAAV 01 (75:25, then 90:10), until the cells are completely transferred to 100% MetaCell® TransAAV 01.
4. Continue culturing in 100% MetaCell® TransAAV 01 for 3-5 passages. When the cell density reaches 3.0-4.0×10^6 cells/mL within 3-4 days of seeding and the cell viability is ≥95%, adaptation is considered complete.
5. Continue passaging at least 3 times. If the cell growth remains stable, subsequent experiments can be conducted.

Transfection

For specific operations, please refer to the user manual of MetaCell® TransAAV Media Panel.

Related Products

Product Name	Classification	Form	Product Code	Size
MetaCell® TransAAV 01	Basal Medium	Powder	P2010-X010	10L
MetaCell® TransAAV Titer Enhancer	Bio-reagent	Liquid	L2013-0010	10mL
			L2013-0100	100mL
			L2013-1000	1000mL
MetaCell® TransAAV Media Panel	kit	Liquid	L2011	1 kit