Cellplus Bio

MetaCell® TransAAV Media Panel

User Manual

Product Description

MetaCell® TransAAV Media Panel is an optimized combination of cell culture media and supplements designed to meet the packaging needs for various AAV (Adeno-Associated Virus) serotypes using multiple HEK293 cell lines. Media in the kit 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 media are highly optimized and compatible with commercial cationic transfection reagents.

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

Product Name	Application
MetaCell® TransAAV 01	MetaCell® TransAAV 01-03 represents a trio of distinct HEK293 culture media formulations, designed to identify the optimal medium that enhances the performance of specific Adeno-Associated Virus (AAV) serotypes, thereby hastening the advancement of cell and gene therapy development.
MetaCell® TransAAV 02	
MetaCell® TransAAV 03	
MetaCell® TransAAV Titer Enhancer	

Cell Culture Conditions

Medium: : MetaCell® TransAAV 01-03

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					

Optimal Transfection Conditions

DNA Volume	FectoVIR-AAV® Volume	DNA to FectoVIR-AAV Ratio (μg: μL)	Volume for Complexes Preparation
3 μg	3 μL	1:1	0.05 mL

Note: The above table lists the amount of transfection complex required per mL of cell culture system.

Recommended Vector Ratios

Vector	μg DNA/mL Culture
Rep/Cap plasmid	1.2
Helper plasmid	0.6
AAV-ITR plasmid	1.2

Note: The above table is calculated based on a total DNA amount of 3 μg.

Media and Reagents

- MetaCell® TransAAV Media Panel
- Recommended transfection reagent: PolyPlus FectoVIR-AAV® Transfection Reagent
- Plasmid: Ratio of 260/280 is among 1.8-2.0 , concentration > 1 μg/μL
- Dilution Solution: MetaCell® TransAAV 01-03 can be used as a dilution solution

Transfection Procedure

- Cell Preparation

1. Three days before transfection, seed cells at a density of 5.0×10^5 cells/mL. Alternatively, seed cells at a density of $2.0\text{-}2.5\times10^6$ cells/mL 18-24 hours before transfection.
2. On the day of transfection, adjust the cell density to the appropriate viable cell density for transfection. The following table provides recommendations:

Cell Line	Transfected Viable Cell Density
Expi293F	3.0×10^6 cells/mL
VPC2.0	3.0×10^6 cells/mL
Other HEK293 Cells	3.0×10^6 cells/mL

- Prepare the Reagent-DNA Mixture

1. Prepare a sterile polypropylene centrifuge tube and add 5% of the cell culture system volume of dilution solution for later use.

   Note: The capacity of the sterile centrifuge tube must be more than 1.5 times the final culture medium volume to provide mixing space.
2. Add DNA to the tube containing the dilution solution and mix evenly (shake or vortex).
3. Add FectoVIR-AAV® reagent under the liquid surface into the tube containing DNA and mix immediately (gently invert and mix 8-10 times).
4. Let the Reagent-DNA mixture stand at room temperature for 30 minutes.

- Transfection

After incubation, add the Reagent-DNA mixture to the shake flask. When dripping, gently shake the shake flask to enhance the mixing of the cell suspension and transfection mixture.

After adding and mixing the transfection complex, add MetaCell® TransAAV Titer Enhancer, with the amount to be

added referenced in the table below:

Working Volume	Cell Line	MetaCell® TransAAV Titer (v/v)
30 mL	Expi293F	1% (300 µL)
	VPC2.0	1% (300 µL)
	其它HEK293细胞	1% (300 µL)

- Cultivation & Harvesting

Virus harvest is conducted 3 days post-transfection.

Related Products

Product Name	Classification	Form	Product Code	Size
MetaCell® TransAAV 01	Basal Medium	Liquid	L2010-0500	500mL
			L2010-1000	1000mL
MetaCell® TransAAV 02	Basal Medium	Liquid	L2007-0500	500mL
			L2007-1000	1000mL
MetaCell® TransAAV 03	Basal Medium	Liquid	L2008-0500	500mL
			L2008-1000	1000mL
MetaCell® TransAAV Titer Enhancer	Bio-reagent	Liquid	L2013-0100	100mL
			L2013-1000	1000mL
MetaCell® TransAAV Media Panel	kit	Liquid	L2011	1 kit