AAV Packaging Mix (Serotype 1)

Cat. No. AAV1001

Store at -20°C.

Product Description

abm's AAV Packaging Mix (Serotype 1) offers a high-efficiency solution for recombinant adeno-associated virus production. This second generation AAV system requires three plasmids for viral particle production: 1) Transfer Plasmid containing your gene of interest, 2) Packaging Plasmid containing replication and capsid (serotype determining) proteins Rep and Cap, and 3) Helper Plasmid containing adenoviral proteins E2A, E4 and VA RNA. The ready-to-use AAV Packaging Plasmid Mix combines optimized Packaging and Helper Plasmids for high viral titers, enhanced biosafety and reliable gene delivery performance.

Packaging Mix Component	Quantity
pAAV-Helper	400 µl, 500 ng/µl
pAAV-R2-C1	

Additional Materials Required (not included)

Material	Recommended Product	Cat. No.
HEK293T Cell Line	293T Cells	LV010
Serum-Free DMEM	PriGrow III Medium	TM003
Transfection Reagent	DNAfectin™ Plus	G2500
Transduction Enhancer	AAViralEntry™ Transduction Enhancer	G516

Protocol

Table 1: Reagent Requirements for AAV Production

Vessel	Seeding Density	DNA Mix			Transfection Mix	
		Transfer Plasmid	Packaging Mix	Serum-Free DMEM	Transfection Reagent	Serum-Free DMEM
15 cm	12.5 x 10^6	20 µg	133 µl	2.5 ml	80 µl	2.5 ml

*Note: the specified amounts and protocol apply to the recommended products and may differ when using alternative products.

Day 1

1. Seed an appropriate number of HEK293T cells into a 15 cm vessel according to Table 1 using complete growth media, and incubate at 37°C with 5% CO_2 overnight.

Day 2

1. Verify that cells have reached 70-80% confluence before proceeding with transfection.
2. Use Table 1 to prepare two solutions, the **DNA Mix** and **Transfection Mix** in 1.5 ml tubes. The DNA Mix includes: Transfer Plasmid, Packaging Mix and Serum-Free DMEM. The Transfection Mix includes: Transfection Reagent and Serum-Free DMEM. Incubate at room temperature for 5 min.
3. Prepare the **Transfection Complex** by combining the DNA Mix and Transfection Mix together, and then incubate at room temperature for 20 min.
4. Add 5 ml of Serum-Free DMEM to the Transfection Complex.
5. Aspirate media from the vessel and gently add the Transfection Complex to the cells. Incubate at 37°C with 5% CO_2 for 5-8 h.
6. Add 5 ml of complete growth media to the vessel and incubate at 37°C with 5% CO_2 overnight.

Day 3

1. Aspirate media from the vessel and add an appropriate amount of complete growth media. Incubate at 37°C with 5% CO_2 for 48 h.

Day 5

1. Harvest cells and media from the vessel using a cell scraper. Collect in a centrifuge tube and centrifuge at 1500 x g for 15 min at 4°C. Discard the supernatant.
2. Wash pellet by adding 2 ml of sterile PBS to the centrifuge tube and centrifuge at 1500 x g for 5 min at 4°C. Discard the supernatant. Repeat Step 2.
3. Add 2 ml of sterile PBS to the centrifuge tube and gently resuspend cells.
4. Lyse cells by performing three consecutive freeze/thaw cycles using a dry ice ethanol bath and a 37°C water bath.
5. Centrifuge at 10,000 x g for 15 min at 4°C.
6. Apply the clarified supernatant over a PES 0.45 µm sterile filter. Use AAV immediately or aliquot into smaller volumes and store at -80°C.
7. Recommended: Add **AAViralEntry™ Transduction Enhancer** to the culture media when using the AAV to enhance downstream transduction efficiency.

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