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1. INTRODUCTION & DESCRIPTION:

The Virongy's AAV Extraction and Maturation Kit simplifies AAV production by providing an efficient solution for crude viral extraction. This kit includes a specialized cell lysis and maturation buffer, enabling high-titer AAV particle recovery with minimal effort. Designed for streamlined workflows, it offers a fast and effective method for extracting AAV from producer cells. The kit's optimized lysis and maturation buffers enhance viral particle recovery, leading to higher titers than conventional freeze-thaw methods. The kit eliminates the need for multiple freeze-thaw cycles, reducing processing time and labor intensity while preserving AAV integrity. Freeze-thaw cycles can cause shear stress and aggregation, reducing functional virus recovery. This kit minimizes degradation, ensuring better particle stability and provides a consistent and scalable extraction process suitable for various AAV serotypes, reducing batch-to-batch variability. The kit streamlines AAV production by removing unnecessary steps, making it ideal for both research and bioprocess development. The AAV Extraction kit provides an efficient, reliable alternative for labs looking to optimize their AAV workflows while achieving superior viral particle recovery.

Highlights:

1. **Higher AAV Yield**
2. **More Efficient Production**
3. **Improved AAV Particle Stability**
4. **Increased Scalability and Reproducibility**
5. **Simplifies Workflow**

2. INTENDED USE:

This product has been manufactured for research use only and is not intended for diagnostic or therapeutic applications in humans or animals. THE PRODUCT IS INTENDED FOR USE ONLY BY PROFESSIONALS TRAINED IN MOLECULAR BIOLOGY AND VIROLOGY TECHNIQUES. This kit is designed for the extraction of crude AAV particles from producer cells using a specialized lysis and maturation buffer system. While optimized for high-titer recovery, performance may vary depending on specific AAV serotypes and production conditions.

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3. KIT COMPONENTS:

VBI AAV Extraction and Maturation Kit	Mini	Midi
Number of AAV Preparations*	10	50
CATALOG no.	AAVEMK-Mini	AAVEMK-Midi
Cell Wash Buffer	8 mL	5 x 8mL
AAV Cell Lysis Buffer	200 µL	5 x 200 µL
AAV Clarification Buffer	200 µL	5 x 200 µL
AAV Elution Buffer	2 mL	5 x 2 mL

AAV Extraction and Maturation Kits should be stored at -20°C
*Each AAV preparation is one transfected well of a 6 well plate

MATERIALS (Not Included)	**Recommended:**
Trypsin treated transfected cell culture/ AAV Producer cells	Transfected HEK293T AAV cell culture production treated with trypsin
Preferred Cell Culture Media or PBS	Gibco, 10010023
Microcentrifuge Tubes	Fisher Scientific, Catalog No.05-408-129
EQUIPMENT:	
Microcentrifuge	

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4. KIT PROCEDURES:

AAV EXTRACTION KIT PROTOCOL (SIX WELL PLATE—35mm WELL):

Before starting: Treat the transfected cell culture with trypsin and neutralize before starting the extraction procedure.
Note: Treating cells with trypsin is important to prevent significant loss of AAV particles.

Extraction Procedure from a 35mm Well:

1. Pellet the trypsin-treated AAV-producing cells and remove the supernatant.
2. Resuspend the pellet in **0.5 mL of Cell Wash Buffer** and transfer to a microcentrifuge tube.
3. Spin the cells at 500 × g for 3 minutes.
4. Remove the supernatant and resuspend the cell pellet in **30 µL** of **Cell Wash Buffer**.
5. Add **10 µL** of **AAV Cell Lysis Buffer** and mix well by gently tapping the tube.
6. Incubate the cell lysate at **37°C for 12–18 hours (overnight)**.
7. Chill the matured lysate for 5 min on ice
8. Clarify the AAV particle containing lysate by adding **10 µL** of **AAV Clarification Buffer**, then mix gently and spin at 5,000 × g for 5 minutes.
9. Transfer the cleared AAV containing supernatant to a new microcentrifuge tube.
10. Elute the remaining AAV from the lysate tube by adding **60 µL** of **AAV Elution Buffer**, then mix gently.
11. Clarify the second eluted lysate by spinning at 5,000 × g for 5 minutes.
12. Transfer this second clarified supernatant to the previously collected cleared supernatant

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AAV EXTRACTION KIT PROTOCOL (100mm PLATE):

Before starting: Treat the transfected cell culture with trypsin and neutralize before starting the extraction procedure.
Note: Treating cells with trypsin is important to prevent significant loss of AAV particles.

Extraction Procedure from a 100mm Plate:

1. Pellet the trypsin-treated AAV-producing cells and remove the supernatant.
2. Resuspend the pellet in **0.5 mL of Cell Wash Buffer** and transfer to a microcentrifuge tube.
3. Spin the cells at 500 × g for 3 minutes**.**
4. Remove the supernatant and resuspend the cell pellet in **140 µL** of **Cell Wash Buffer**.
5. Add **50 µL** of **AAV Cell Lysis Buffer** and mix well by gently tapping the tube.
6. Incubate the cell lysate at 37°C for 12–18 hours (overnight)**.**
7. Chill the matured lysate for 5 min on ice
8. Clarify the AAV particle containing lysate by adding **50 µL** of **AAV Clarification Buffer**, then mix gently and spin at 5,000 × g for 5 minutes.
9. Transfer the cleared AAV containing supernatant to a new microcentrifuge tube.
10. Elute the remaining AAV from the lysate tube by adding **500 µL** of **AAV Elution Buffer**, then mix gently.
11. Clarify the second eluted lysate by spinning at 5,000 × g for 5 minutes**.**
12. Transfer this second clarified supernatant to the previously collected cleared supernatant

FINAL NOTES:

- The cleared AAV-containing supernatant is a crude extraction that can be used directly in cell culture experiments by diluting 1:50 with PBS or cell culture media. Dilute more as needed to avoid cytotoxicity.
- Alternatively, the supernatant can be further purified using the AAV Column Purification Kit**.**

STORAGE INSTRUCTIONS:

- The cleared AAV-containing supernatant can be stored at 4°C for up to 1 week.
- For long-term storage, transfer the supernatant to -80°C.
- If the AAV is diluted (e.g., for cell culture use), store at -80°C to maintain stability.