AAV-MAX Lysis Buffer

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ AAV-MAX Lysis Buffer is a ready-to-use, chemically defined, Polysorbate 20-based cell lysis reagent for the extraction of AAV particles from producer HEK293 cells. The buffer is supplied as a 10X solution that can be directly added to HEK293 AAV production cultures to induce cell lysis.

Contents and storage

Product	Cat. No.	Amount	Storage
Gibco™ AAV-MAX Lysis Buffer	A50520	100 mL	2°C to 8°C, protect from light

Procedural guidelines

- The AAV-MAX Lysis Buffer is a 10X formulation that can be added directly to the cell culture to induce lysis.
- Handling of AAV particles must be performed as per institutional guidelines.
- All materials that come into contact with AAV solution should be appropriately disinfected prior to disposal.
- Harvest AAV particles 70 to 72 hours post-transfection.

Harvest AAV particles

1. Add AAV-MAX Lysis Buffer directly to the culture flask at a 1:10 dilution (e.g., 3.3 mL of AAV-MAX Lysis Buffer to a 30 mL culture volume) and swirl the flask to evenly distribute the lysis buffer.

   Note: If performing qPCR measurement of titers, remove 5 mL from the culture flask after performing step 1 and perform lysis by incubating at 37°C for 1 hour at 250 rpm on an orbital shaker. Proceed to step 3.

   Note: If proceeding to a downstream workflow, add $MgCl_2$ (final concentration: 2 mM) and Benzonase (final concentration: 90 U/mL) to the remaining culture in the flask from step 1, then proceed to step 2.

2. Incubate the flask at 37°C for at least 2 hours on an orbital shaker (for suggested shake speeds, see Table 1).

   Note: Once cells are lysed, the culture will appear to contain visible cell debris.

Note: Lysis incubation times can vary based on AAV serotype and production scale. Therefore, it is recommended to optimize lysis conditions for your experiments prior to moving to large scale.

Table 1 Recommended volumes for routine cell culture maintenance

Flask size	Culture volume[1]	Shake speed
125 mL	30 mL	125 ± 5 rpm (19-mm orbital diameter) 120 ± 5 rpm (25-mm orbital diameter) 95 ± 5 rpm (50-mm orbital diameter)
250 mL	60 mL	
500 mL	120 mL	
1 L	240 mL	
2 L	480 mL	
2.8 L	700 –1000 mL	90± 5 rpm (19-mm orbital diameter) 85± 5 rpm (25-mm orbital diameter) 80± 5 rpm (50-mm orbital diameter)

[1] If using volumes outside of the recommended range, it is critical to ensure that all cell growth (i.e., doubling times), health (i.e., cell diameter, viability), and expression levels remain consistent with control conditions. Cell performance is decreased if cell health is compromised.

3. Transfer the cell lysate to an appropriate flask or tube, then centrifuge at 4°C at 13,000 × *g* for 10 min for smaller volumes (microfuge scale) or at 4,500 × *g* for 30 minutes for larger scale of production.

4. Transfer the supernatant containing crude AAV particles to an appropriate storage container.

5. For qPCR titer measurement, transfer 50 to 100 µL of lysate to a 96-well plate.

   Note: It is recommend that multiple replicates for each sample are prepared to account for qPCR assay variation.

6. Harvested crude AAV particles can be stored at -80°C for long term storage.

   Note: To avoid repeated freeze/thaw cycles, virus aliquoting is highly recommended. To thaw AAV samples, bring the tube to room temperature and mix the AAV sample by gentle pipetting or inverting. Do not vortex and avoid mixing vigorously.

   Note: Crude AAV vectors can temporarily be stored at 4°C for short duration (i.e. overnight). If samples are stored for extended periods of time at 4°C, precipitation can occur. This is dependent on AAV serotype and/or production scale. If precipitation is observed, reclarify the samples according to step 3 prior to proceeding to the next step.

   Note: To disinfect AAV properly, prepare a 10% bleach solution, then disinfect used pipette tips, serological pipettes and culture flasks before disposal. To discard remaining AAV samples, add the 10% bleach solution directly to the AAV solution, then incubate for a minimum of 30 minutes before disposal.

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