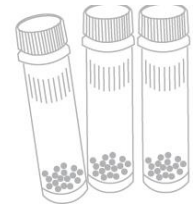
CRUDE CELL LYSATE PREPARATION FOR RECOMBINANT ADENOVIRUS-ASSOCIATED VIRUS (RAAV) VECTOR PRODUCTION



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CONTEXT

Traditionally, in order to release intracellular retained rAAV vectors, producer cells are lysed by 3 to 4 freeze-thaw-cycles (liquid nitrogen-37 °C water), producing a crude cell lysate that is the starting material for a variety of subsequent purification and concentration methods. Therefore, efficient cell lysis is of crucial importance towards high-titer rAAV vector preparations. This application note investigates the use of the Minilys as an alternative solution to traditional freeze-thaw methods.

MATERIALS

- **Minilys homogenizer**
- Lysing kits: CK14 2mL (KT03961-1-003.2) and CK14 7mL (KT03961-1-307.7)
- Samples: 15cm cell culture dishes containing HEK 293T producer cells (to be lysed), either untransfected (self-complementary (sc) rAAV vectors containing a CMV promoter-driven EGFP expression cassette added before lysis) or transfected (producing scrAAV vectors containing a CMV promotor driven DsRed2 monomer expression cassette before lysis)
- Buffer: AAV resuspension buffer (50 mM Tris-HCl, pH 8.5, 150 mM NaCl)

PROTOCOL

- Untreated HEK 293T producer cells from one confluent 15cm cell culture dish (> 2x10E7 cells) were resuspended in 1.5 ml AAV buffer and supplemented with 10 µl of serotype 2 scrAAV-2/2-CMV-EGFP vector (1x10E13 vector genomes (vg)/ml)), followed by homogenization on the Minilys. **Parameters used** were 5000 rpm, 2 cycles of 60 seconds each (30 second pause between cycles) with CK14 2mL lysing tubes **(Figure 1A)**.
- Transfected HEK 293T producer cells (producing scrAAV-2/2-CMV-DsRed2 monomer) were collected from five confluent 15cm cell culture dishes 3 days post-transfection and resuspended in 5 ml AAV buffer, followed by homogenization on the Minilys. **Parameters used** were 5000 rpm, 3 cycles of 40 seconds each, with CK14 7mL lysing tubes **(Figure 2A).**

RESULTS

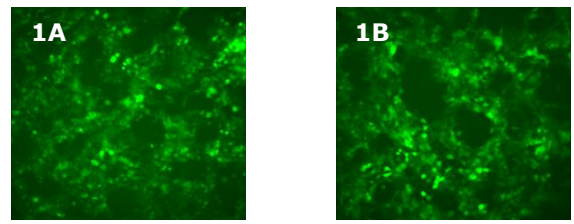

Figure 1. Minilys homogenization (A) *vs.* 3 freeze-thaw cycles (B) on untransfected HEK 293T cells resuspended with known concentrations of rAAV vector titers. 700 µl of the crude cell lysate obtained from each method was added to separate dishes of cultured HEK 293T cells. Images were taken 2 days later (20x objective).

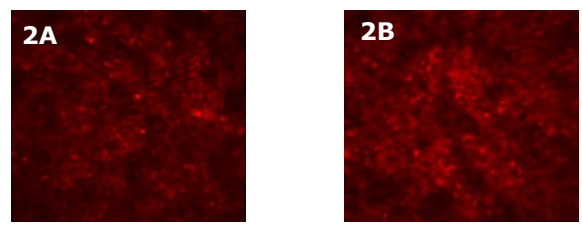

Figure 2. Transduction of HEK 293T cells by rAAV vectors produced by transient transfection of HEK 293T cells and released thereafter by Minilys homogenization (A) vs. 4 freeze-thaw cycles (B). 10 µl of the crude cell lysate obtained from each method was added to separate dishes of cultured HEK 293T cells. Images were taken 6 days later (20x objective).

The images show that the **Minilys** does not impair rAAV vector infectivity (Figure 1) and efficiently releases rAAV vectors from HEK 293T producer cells (Figure 2), and thus shows similar results compared to the freeze-thaw method.

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CONCLUSION

The Minilys homogenizer in combination with the CK14 2mL and 7mL lysing tubes offers a safe, fast and efficient way to release rAAV vectors from HEK 293T producer cells, without impairing infectivity. Therefore, the Minilys is a valuable alternative to the traditional method of freezing and thawing, and also saves a significant amount of sample preparation time.

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