

Materials List for:

# A Quantitative Dot Blot Assay for AAV Titration and Its Use for Functional Assessment of the Adeno-associated Virus Assembly-activating Proteins

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URL: <https://www.jove.com/video/56766>

DOI: [doi:10.3791/56766](https://doi.org/10.3791/56766)

## Materials

| Name                                                           | Company                       | Catalog Number  | Comments                                                                                                                                                                                                |
|----------------------------------------------------------------|-------------------------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Benzonase                                                      | MilliporeSigma                | 1016970001      | Referred to as <i>Serratia marcescens</i> endonuclease in the main text.                                                                                                                                |
| DNase I                                                        | Roche                         | 4716728001      | Referred to as DNase I Enzyme A in the main text.                                                                                                                                                       |
| DNase I                                                        | Invitrogen                    | 18047019        | Referred to as DNase I Enzyme B in the main text.                                                                                                                                                       |
| DNase I                                                        | New England Biolabs           | M0303L          | Referred to as DNase I Enzyme C in the main text.                                                                                                                                                       |
| 1.5 mL Attached O-Ring Screw Cap Microcentrifuge Tubes         | Corning                       | 430909          |                                                                                                                                                                                                         |
| 1.5 mL Microcentrifuge Tubes                                   | Thermo Fisher Scientific      | 05-408-129      |                                                                                                                                                                                                         |
| 15 mL Polypropylene Conical Tube                               | Corning                       | 352097          |                                                                                                                                                                                                         |
| 3 M Sodium Acetate                                             | Teknova                       | S0298           |                                                                                                                                                                                                         |
| 50 mL Polypropylene Conical Tube                               | Corning                       | 430291          |                                                                                                                                                                                                         |
| AAV-293 Cells                                                  | Agilent                       | 240073          | HEK-293 cell line optimized for the packaging of AAV virions.                                                                                                                                           |
| AccuGENE 0.5 M EDTA Solution                                   | Lonza                         | 51234           |                                                                                                                                                                                                         |
| AccuGENE 1 M Tris-HCl pH 8.0                                   | Lonza                         | 51238           |                                                                                                                                                                                                         |
| AccuGENE 10% SDS                                               | Lonza                         | 51213           |                                                                                                                                                                                                         |
| AccuGENE 1X TE Buffer                                          | Lonza                         | 51235           |                                                                                                                                                                                                         |
| Bio-Dot Apparatus                                              | Bio-Rad                       | 1706545         |                                                                                                                                                                                                         |
| Bovine Serum Albumin                                           | MilliporeSigma                | A3294           |                                                                                                                                                                                                         |
| Cell Lifter                                                    | Corning                       | 3008            |                                                                                                                                                                                                         |
| Chloroform                                                     | MilliporeSigma                | 372978          |                                                                                                                                                                                                         |
| DNA Extractor Kit                                              | Wako Pure Chemical Industries | 295-50201       | This is used for quantitative dot blots on purified virus. We use only a half volume of all the reagents in each step recommended for the Protocol Scheme 2 in the manual provided by the manufacturer. |
| Dulbecco's Modified Eagle Medium (DMEM)-high glucose (4.5 g/L) | Lonza                         | 12-614F         |                                                                                                                                                                                                         |
| ElectroMAX DH10B Cells                                         | Thermo Fisher Scientific      | 18290015        |                                                                                                                                                                                                         |
| Ethanol 200 Proof                                              | Decon Labs                    | 2716            |                                                                                                                                                                                                         |
| Fetal Bovine Serum                                             | VWR                           | 1500-500        |                                                                                                                                                                                                         |
| Ficoll 400                                                     | Alfa Aesar                    | B22095          | Referred to as Polysucrose 400.                                                                                                                                                                         |
| Fluorescence Microscope                                        | Zeiss                         | Axiovert 40 CFL |                                                                                                                                                                                                         |

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|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-------------------------------------|--|
| Glycogen                                                                                                                                                                                         | Roche                       | 10901393001                         |  |
| Herring Sperm DNA                                                                                                                                                                                | Invitrogen                  | 15634-017                           |  |
| Hybridization Tubes                                                                                                                                                                              | Thermo Fisher Scientific    | 13-247-150                          |  |
| ImageQuant TL                                                                                                                                                                                    | GE Healthcare Life Sciences | ImageQuant TL                       |  |
| L-glutamine 200 mM                                                                                                                                                                               | Lonza                       | 17-605E                             |  |
| Magnesium Chloride Hexahydrate                                                                                                                                                                   | MilliporeSigma              | M0250                               |  |
| MicroPulser Electroporator                                                                                                                                                                       | Bio-Rad                     | 1652100                             |  |
| Mini Quick Spin DNA Columns                                                                                                                                                                      | MilliporeSigma              | 11814419001                         |  |
| pAAV-RC2 Vector                                                                                                                                                                                  | Cell Biolabs Inc            | VPK-422                             |  |
| Penicillin/Streptomycin 10K/10K                                                                                                                                                                  | Lonza                       | 17-602E                             |  |
| Phosphate Buffered Saline                                                                                                                                                                        | Lonza                       | 17-516F                             |  |
| Phosphorimaging Exposure Cassette                                                                                                                                                                | GE Healthcare Life Sciences | Various                             |  |
| Phosphorimaging Screen                                                                                                                                                                           | GE Healthcare Life Sciences | Various                             |  |
| Plasmid Maxi Kit                                                                                                                                                                                 | QIAGEN                      | 12162                               |  |
| Platinum Pfx DNA Polymerase                                                                                                                                                                      | Invitrogen                  | 11708013                            |  |
| Polyethylenimine                                                                                                                                                                                 | Polysciences Inc            | 23966-2                             |  |
| Polyvinylpyrrolidone                                                                                                                                                                             | MilliporeSigma              | P5288                               |  |
| Prime-It II Random Primer Labeling Kit                                                                                                                                                           | Agilent Technologies        | 300385                              |  |
| Primer AAP Forward (N <sub>25</sub> nucleotides from the 4th nucleotide in the AAP ORF, RE1 is a restriction enzyme site for cloning): CTAA-RE1-CACCATGGACTACAAGGA CGACGATGACAAA-N <sub>25</sub> | MilliporeSigma              | Custom Primer                       |  |
| Primer AAP Reverse (N <sub>25</sub> is the last 25 nucleotides of the AAP ORF, RE2 is a restriction enzyme site for cloning): TCTT-RE2-N <sub>25</sub>                                           | MilliporeSigma              | Custom Primer                       |  |
| Primer VP3 Forward (N <sub>25</sub> the first 25 nucleotides of the VP3 ORF, RE1 is a restriction enzyme site for cloning): CTAA-RE1-CACC-N <sub>25</sub>                                        | MilliporeSigma              | Custom Primer                       |  |
| Primer VP3 Reverse (N <sub>25</sub> is the last 25 nucleotides of the AAP ORF, RE2 is a restriction enzyme site for cloning): TCTT-RE2-N <sub>25</sub>                                           | MilliporeSigma              | Custom Primer                       |  |
| Proteinase K Solution                                                                                                                                                                            | Invitrogen                  | 25530-049                           |  |
| Restriction Enzymes                                                                                                                                                                              | New England Biolabs         | Various                             |  |
| Salmon Sperm DNA                                                                                                                                                                                 | Invitrogen                  | 15632011                            |  |
| Serological Pipettes                                                                                                                                                                             | Thermo Fisher Scientific    | 13-678-11D / 13-678-11E / 13-678-11 |  |
| Sodium Chloride                                                                                                                                                                                  | Thermo Fisher Scientific    | S271-10                             |  |
| Sodium Citrate Tribasic Dihydrate                                                                                                                                                                | MilliporeSigma              | C8532                               |  |
| T4 DNA Polymerase                                                                                                                                                                                | New England Biolabs         | M0203L                              |  |
| Thermal Cycler                                                                                                                                                                                   | Eppendorf                   | 6321000515                          |  |
| Tissue-culture Treated 6-well Plate                                                                                                                                                              | Corning                     | 353046                              |  |
| Tris Base                                                                                                                                                                                        | Thermo Fisher Scientific    | BP152-5                             |  |
| Typhoon FLA 7000                                                                                                                                                                                 | GE Healthcare Life Sciences | 28955809                            |  |

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|-----------------------------------|-------------|-----------|------------------------------------------------------------------------------------------|
| UltraPure Buffer-Saturated Phenol | Invitrogen  | 15513-047 |                                                                                          |
| UV Crosslinker                    | Spectroline | XLE-1000  | Optimal Crosslink mode is used, providing a UV energy dosage of 120 mJ/cm <sup>2</sup> . |
| Zeta-Probe Membrane               | Bio-Rad     | 162-0165  |                                                                                          |