Electronic Supplementary Information for

Adeno-associated viral vector purification using a centrifugal microfluidic system: Towards workflow automation for low-volume sample processing

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Table S1 Buffer solutions used in the AAV purification process

Buffer	Composition	pH	Supplier
EqB	20 mM Tris, 150 mM trisodium citrate dihydrate	7.2	BioShop (Burlington, ON), Sigma-Aldrich
EB	100 mM glycine	2.5	BioShop
NB	1 M Tris	8.8	BioShop
DB	20 mM Tris, 150 mM trisodium citrate dihydrate	7.2	BioShop, Sigma-Aldrich

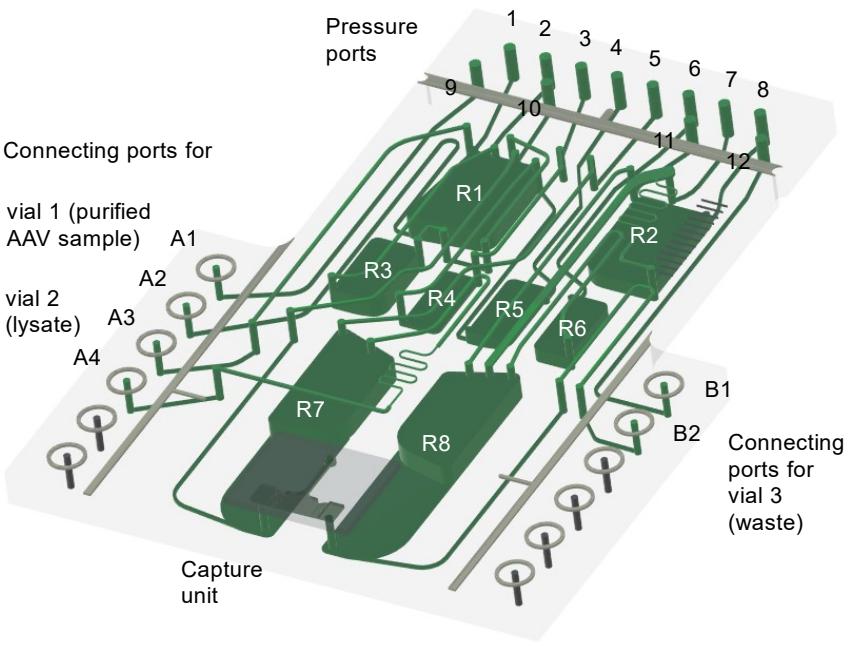

Fig. S1 Configuration of the microfluidic circuit (3D view) implemented on the cartridge.

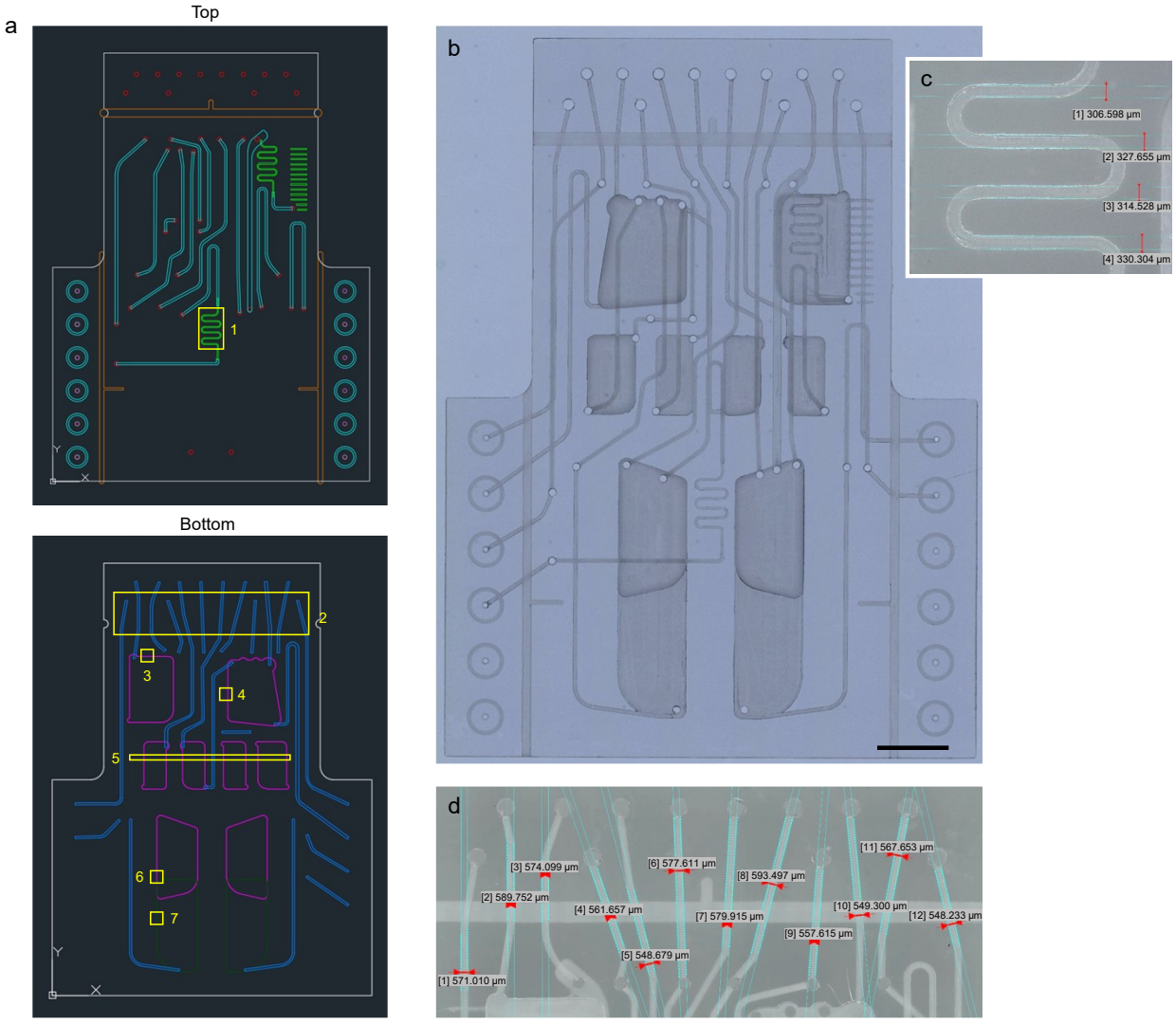

Fig. S2 Two-level microfluidic circuit. (a) CAD file of the structures implemented on the top and bottom side of the PC part. Note, that the bottom view mirrors the microfluidic configuration seen from the top. Regions marked in yellow were used to perform metrology after CNC machining (Table S2). (b) Optical microscopy image of a machined PC part (top view). Scale bar: 1 cm. (c) Close-up view of the serpentine channel. Straight channel segments were used for width measurements. (d) View of the upper portion of the cartridge (bottom side) used to determine channel width.

Table S2 Metrology of fluidic design elements produced by CNC machining

Inspection zone	Unit	Design		Machined PC part	
		Width (μm)	Depth (μm)	Width (μm)	Depth (μm)
1	Serpentine channel	260	160	320[a]	263
2	Transfer channels	520	300	568[b]	503
3	R2		3,000		3,066
4	R1		4,000		3,946
5	R3		3,000		3,031
	R4		500		581
	R5		2,000		2,082
	R6		2,000		2,089
6	R8, upper portion		3,000		3,102
7	R8, lower portion		300		344

[a] Average of four measurements (Fig. S2c). [b] Average of 12 measurements (Fig. S2d).

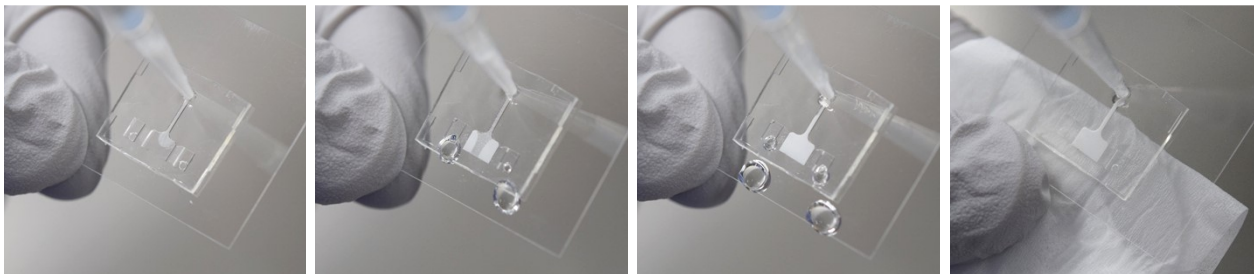

Fig. S3 Filling of the capture unit. A diluted suspension of bead slurry is pipetted into the filling port. Beads accumulate gradually while excess NaCl solution is leaving through the constriction area implemented on the left- and right-hand side of the capture chamber. Once filling is completed, the back side of the unit is dried using laboratory wipe.

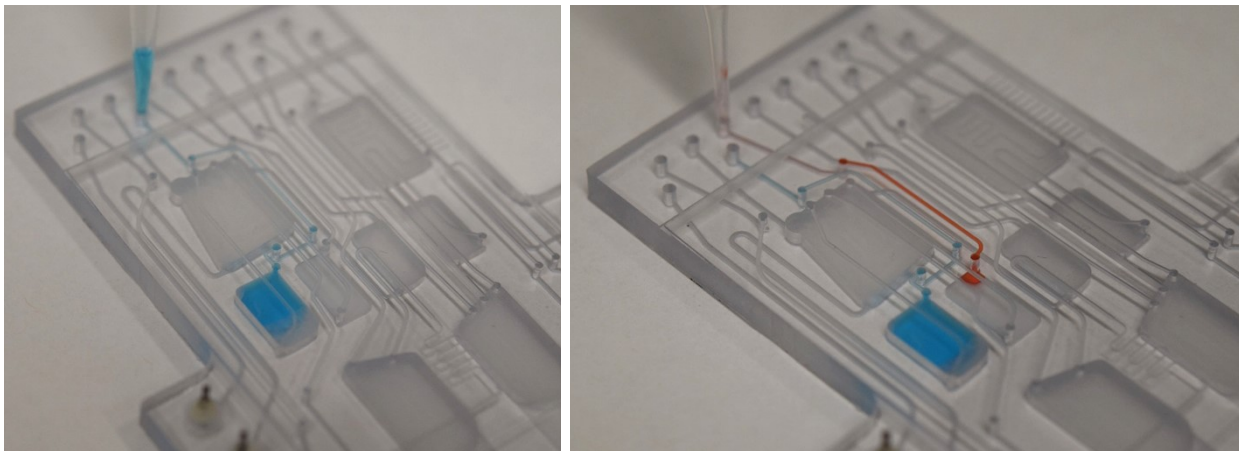

Fig. S4 Filling of reservoirs on the microfluidic cartridge using a micropipette. Left-hand panel: 190 µL of blue-colored solution is pushed into reservoir R3 (through pressure port #10). Right-hand panel: 10 µL of red-colored solution is pushed into reservoir R4 (through pressure port #3).

Table S3 Probe and primer sequences[a] used in the pcDNA3-EGFP qPCR assay

Name	Sequence (5′ → 3′)
Forward primer	GCA CAA GCT GGA GTA CAA CTA
Reverse primer	TGT TGT GGC GGA TCT TGA A
Probe	5HEX/AGCAGAAGA/ZEN/ACGGCATCAAGGTGA/3IABkFQ

[a] Obtained from Integrated DNA Technologies (Coralville, IA).

Table S4 Dilution series for pcDNA3-EGFP and corresponding Cq values

Standard	[EGFP] (ng/μL)	log[EGFP]	Cq (average)[a]
1	0.00025	–3.6021	24.9400
2	0.0025	–2.6021	22.3100
3	0.025	–1.6021	18.5000
4	0.25	–0.6021	16.2500
5	25	1.3979	11.1500

[a] Obtained from two measurements (n = 2).

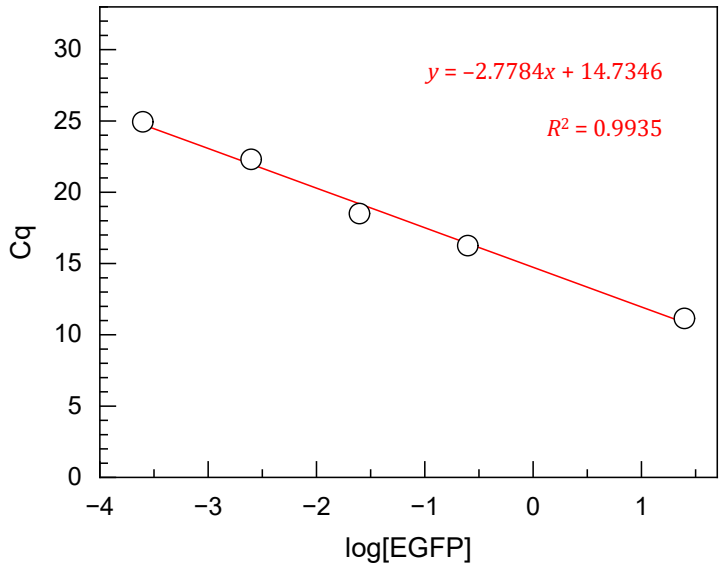

Fig. S5 Standard curve used for quantification of EGFP. The line represents a linear fit of the data set.

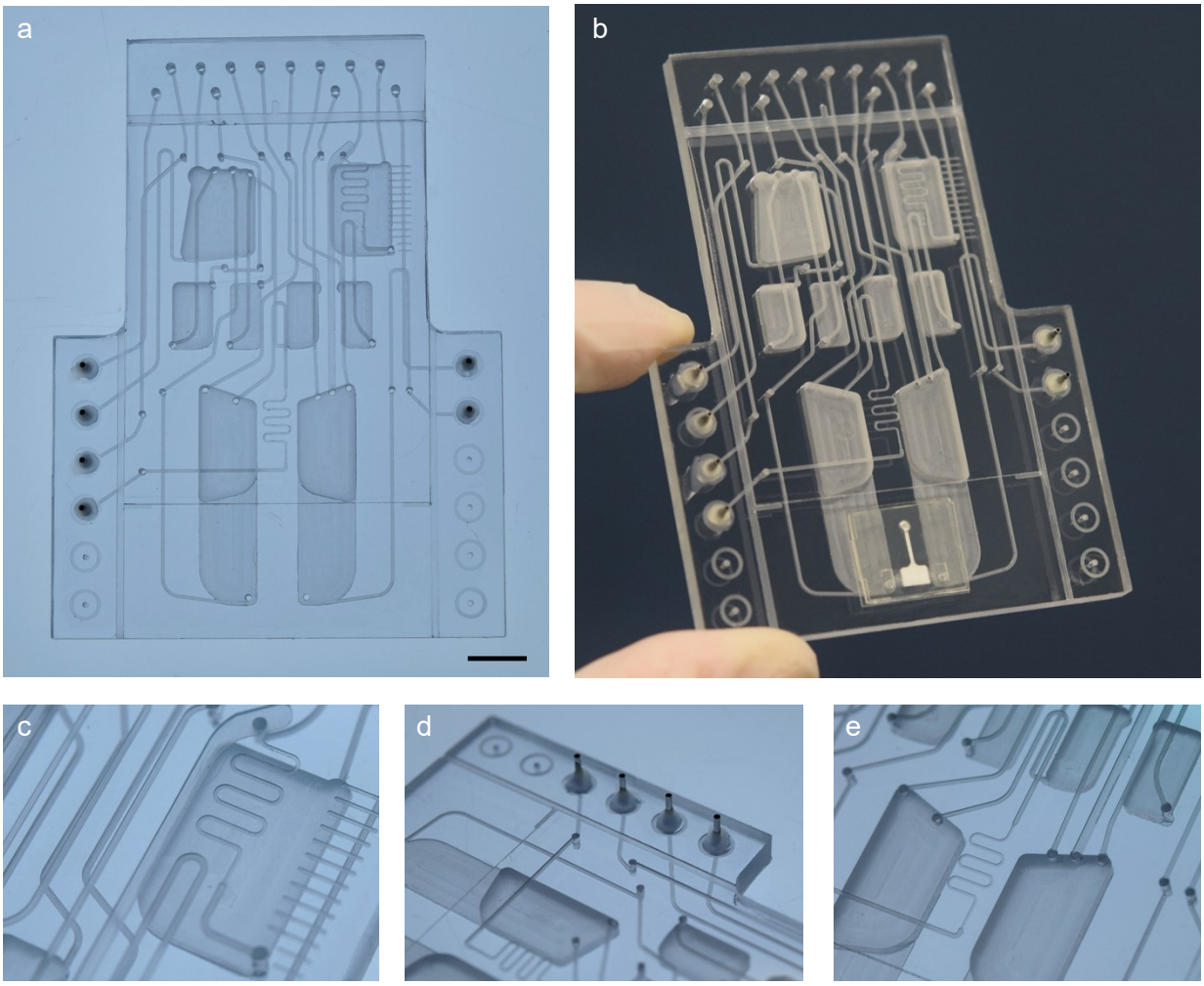

Fig. S6 Microfluidic cartridge. (a) Photograph (top view) after reservoirs have been sealed on the top and bottom of the machined PC part. Connectors were inserted into the respective ports on the left- and right-hand side of the fluidic circuit. Scale bar: 1 cm. (b) Photograph (3D view) of the cartridge with the capture unit put in place. (c–e) Close-up views showing different segments of the microfluidic circuit.

Table S5 Designation of reservoirs on the microfluidic cartridge

Reservoir	Buffer (initial stage)	Buffer volume (µL)	Dedicated pressure port #
R1	n.a.	–	2
R2	EqB	200	8
R3	DB	190	10
R4	NB	10	3
R5	EqB	100	4
R6	EB	100	6
R7	n.a.	–	5
R8	n.a.	–	7

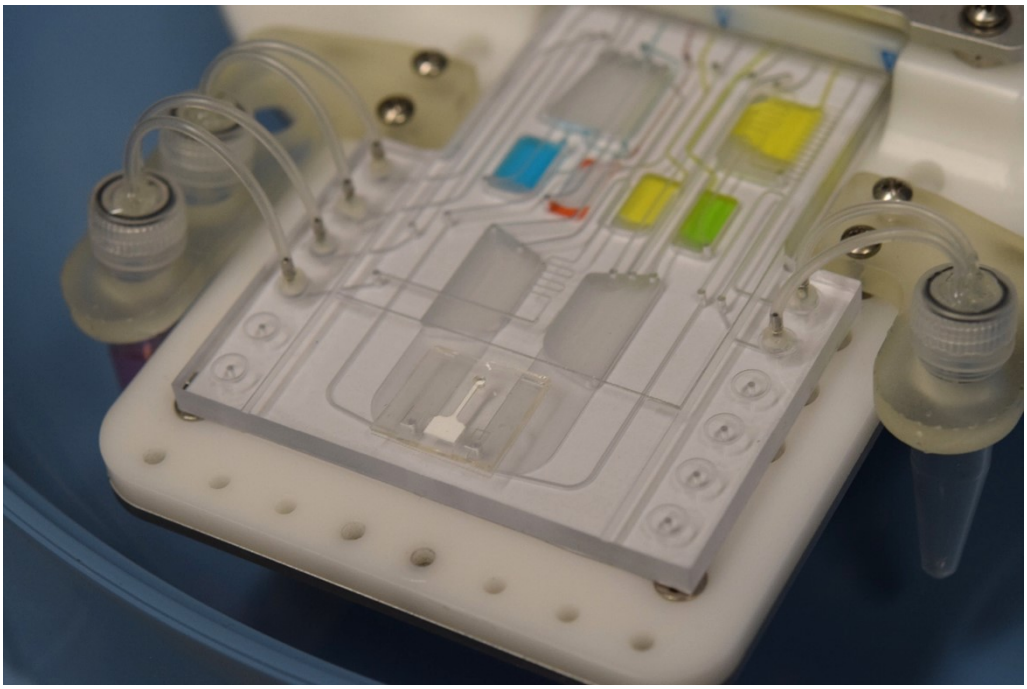

Fig. S7 Microfluidic cartridge on the rotating stage. Pressure ports (located on the upper portion of the cartridge) are sealed by the manifold lid (for which the vertical position can be adjusted manually). External vials are held in place using 3D-printed support structures. Colored solutions were inserted into reservoirs for demonstration purposes. Liquid remaining in channels used in the filling process will be pushed down into the respective reservoirs once the platform starts rotating.

Table S6 External vials used in the on-chip AAV purification process

Vial	Purpose	Connecting ports	Function
1	Collect purified AAV sample	A1	Application of negative pressure
		A2	Liquid transfer from R1
2	Provide crude lysate	A3	Application of positive pressure
		A4	Liquid transfer to R7
3	Collect waste	B1	Application of negative pressure
		B2	Liquid transfer from R8

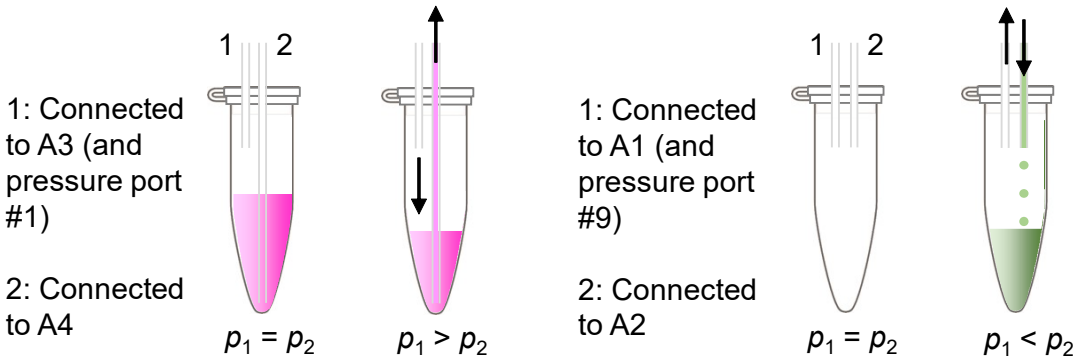

Fig. S8 Liquid transfer to and from external vials. The left-hand panel illustrates connectivity for vial 2 containing crude lysate. Tubing (1) is cut short while tubing (2) extends to the bottom of the vial. When the tube is pressurized (by applying positive pressure to port #1), liquid is pushed up in tubing (2) and so can be transferred to R7 on the cartridge. The right-hand panel depicts connectivity for vial 1 destined to collect the purified sample. Tubing (1) and (2) are both trimmed. Applying negative pressure to port #9 causes liquid to be aspirated into the exit channel connected to reservoir R1 and further into the vial.

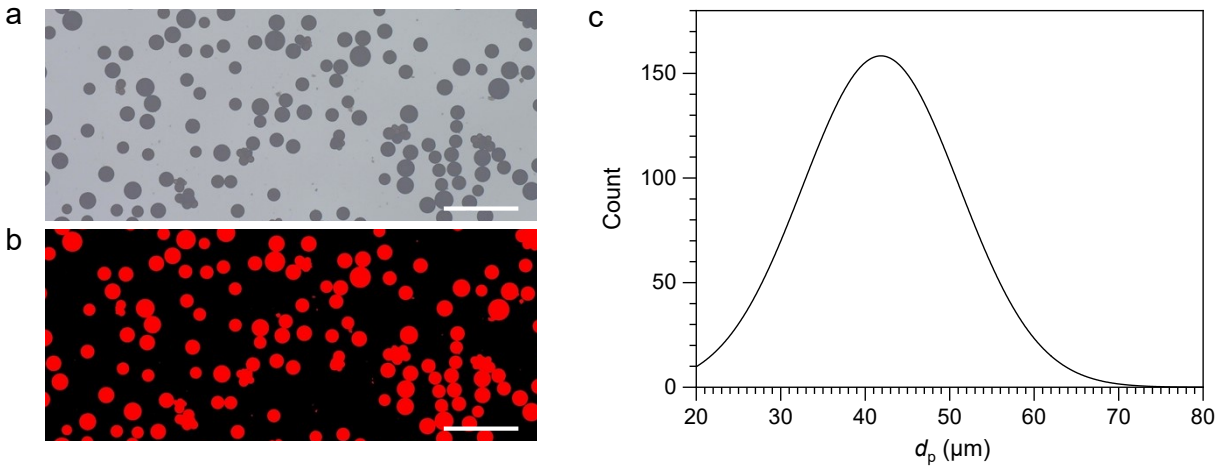

Fig. S9 Assessment of particle size distribution for POROS™ CaptureSelect™ AAVX Affinity Resin. (a) Optical micrograph of diluted bead slurry. (b) Binary image used for particle analysis. Scale bars: 200 µm. (c) Plot of the particle size distribution derived from the experimental data set (n = 736). The mean value has been calculated as d_p = 41.9 ± 9.3 µm.

Table S7 Assessment of pcDNA3-EGFP removal for on-chip sample purification

Sample	Cq (average)[a]	log[EGFP]	[EGFP] (ng/μL)	Removal (%)
A	29.67	–5.3753	4.21×10^{-6}	99.993
B	29.71	–5.3897	4.08×10^{-6}	99.993
C	30.98	–5.8468	1.42×10^{-6}	99.998

[a] Obtained from two measurements (n = 2).