Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Isolation of pathogenic bacteria from sputum samples using a 3D-printed cartridge system

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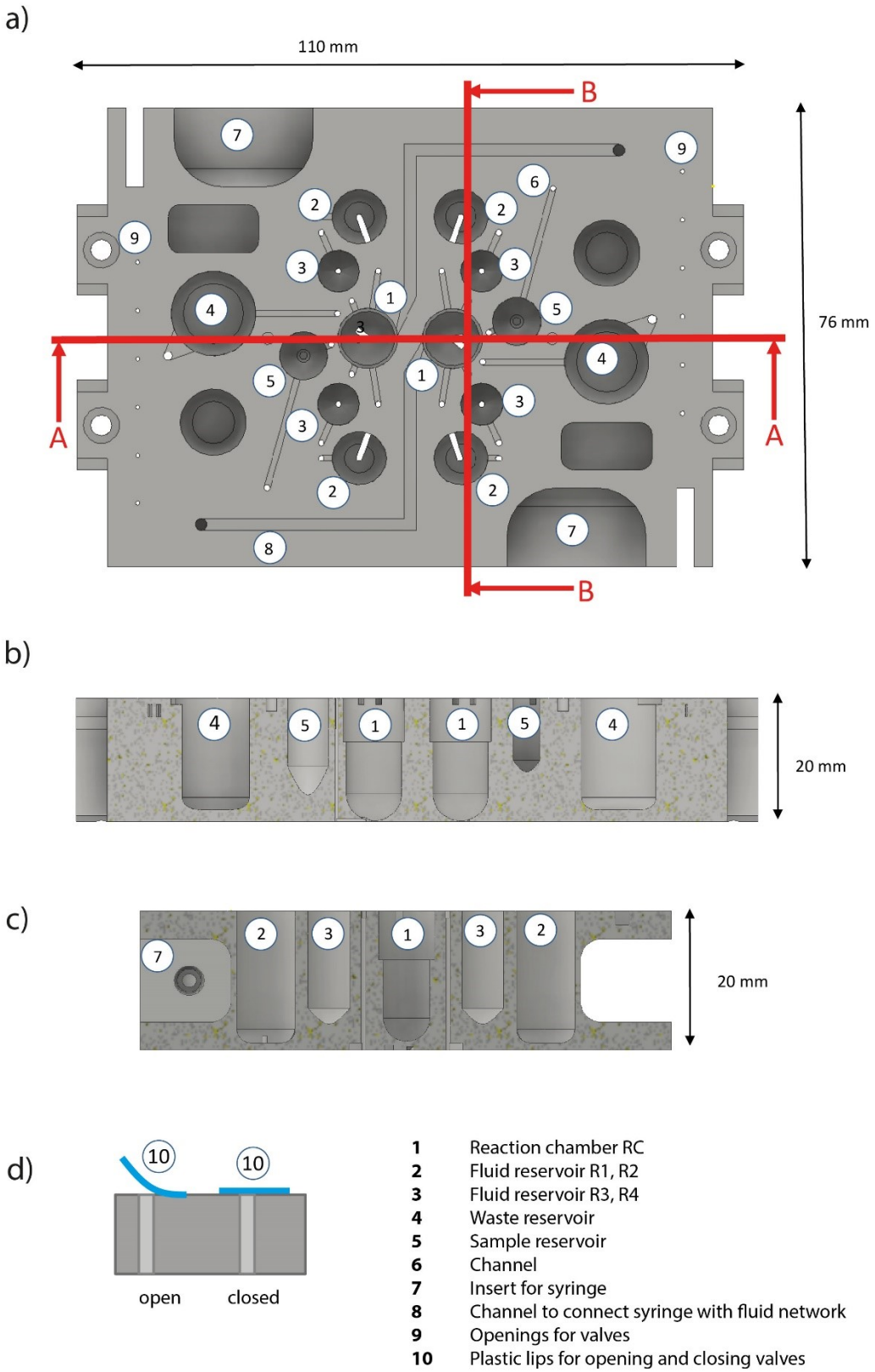

Figure S1. Schematic display of the cartridge design: a) top view, b) sectional view A-A, c) sectional view B-B, d) operating principle of the valves.

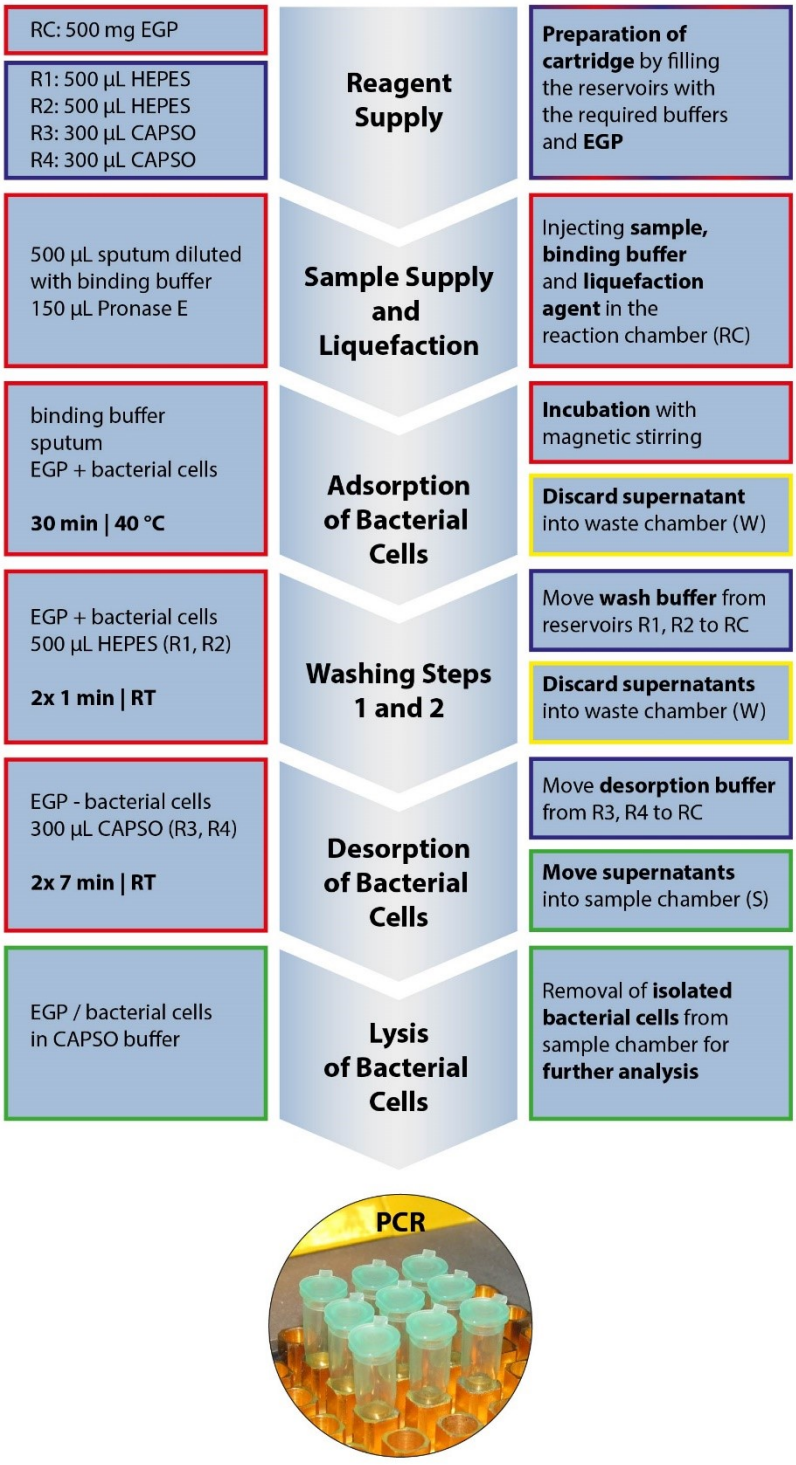

Figure S2. Flow chart illustrating the processing steps performed within the cartridge.

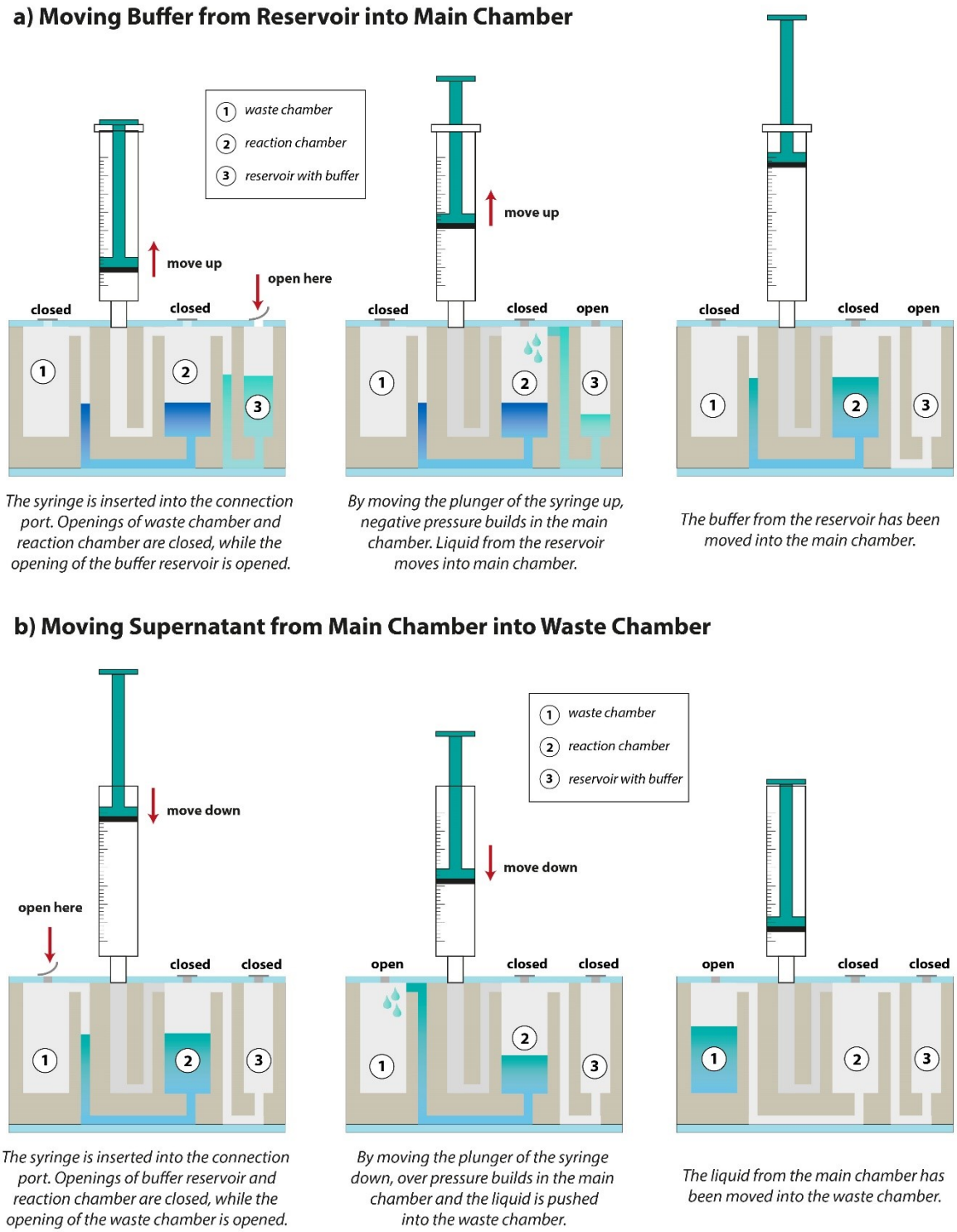

Figure S3. Step by step illustration of the working principle of the cartridge for two operations. a) Moving Buffer from a reservoir into the main chamber. b) Moving supernatant from the main chamber into the waste chamber.

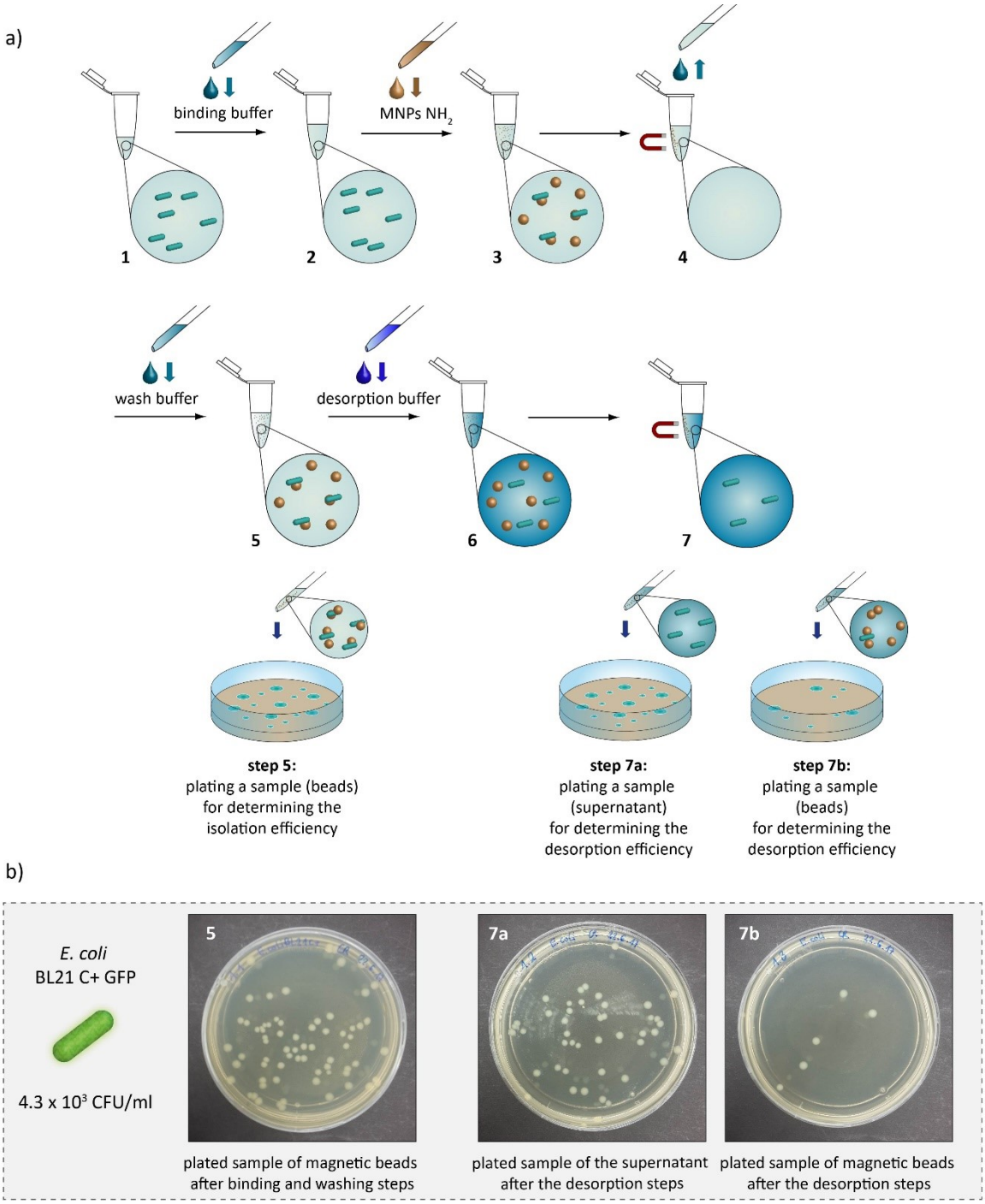

Figure S4. a) Schematic display of the isolation protocol using amine modified magnetic beads: **(1)** sample with bacteria **(2)** after adjusting the pH value by adding binding buffer **(3)** after incubation the bacterial cells have bound to the beads **(4)** using magnetic separation, the supernatant is removed while the bead bound bacteria remain in the tube **(5)** washing steps are performed for removing any undesired residues from the sample **(6)** by raising the pH value through adding desorption buffer the bacteria are detached from the bead surface **(7)** using magnetic separation the beads can be separated from the liquid phase, which contains the bacterial cells in buffer solution b) Exemplary plates of a sample with *E. coli* after different steps in the protocol for determining the isolation efficiency. **(Step 5)** After the bacterial cells are bound to the beads and have been washed a diluted sample containing the magnetic beads is plated for determining the isolation efficiency. **(Step 7a** and **7b)** After the bacterial cells have been removed from the beads samples of the supernatant and the beads are plated separately for checking how efficiently the cells can be removed from the bead surface. Theoretical the CFU numbers of 7a and 7b should yield the CFU count of 5a, because equal volumes were plated.

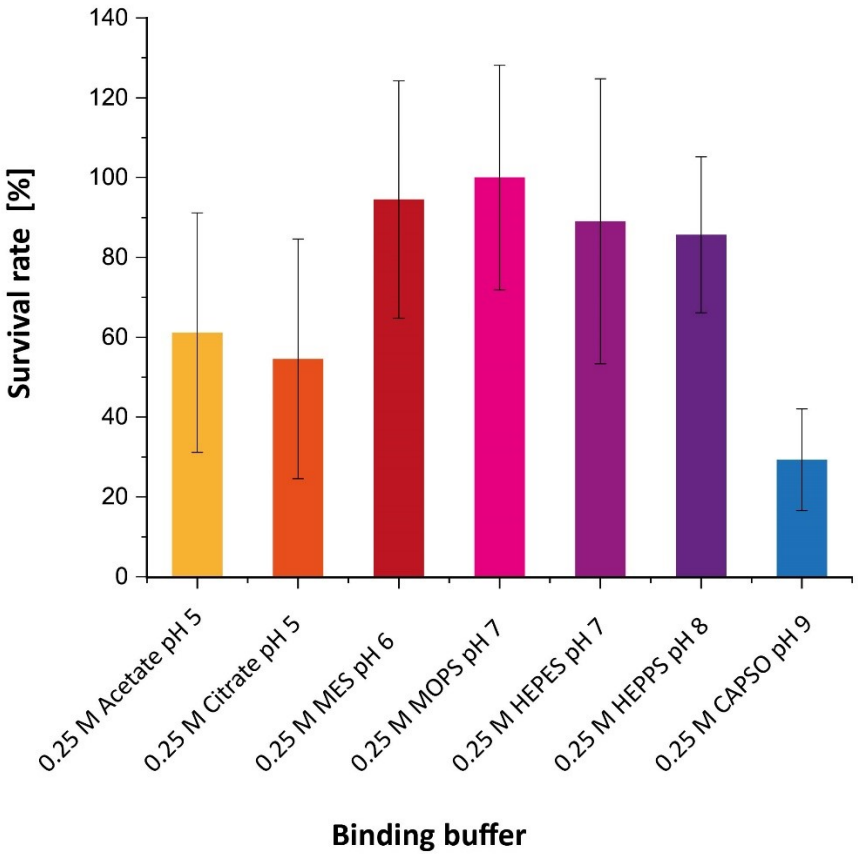

Figure S5. Survival rates of bacteria in different binding buffer types. Equal volumes of a liquid culture of *E. coli* BL21 C+ in the exponential growth phase were added to different binding buffers to a concentration of approximately 3.5 x 10^4 CFU/ml and incubated for 30 min. Subsequently, equal volumes of the buffer samples were plated and incubated over-night. The survival rate was determined by comparing the average CFU numbers to the CFU count of the buffer yielding the highest number of CFU (0.25 M MOPS = 100 %).

Table S1. Total yields calculated in CFU/ml and survival rates of bacteria relative to the CFU count of the buffer with the maximum number of CFU (0.25 M MOPS = 100 %). (See caption of Figure S2 for more experimental details.)

	Yield [CFU/ml]		**Survival rate [%]**	
	Mean value	*Standard deviation*	*Mean value*	*Standard deviation*
0.25 M Acetate pH 5	9.33 x 10^3	3.11 x 10^3	61%	30%
0.25 M Citrate pH 5	8.33 x 10^3	2.78 x 10^3	55%	30%
0.25 M MES pH 6	1.44 x 10^4	4.54 x 10^3	95%	30%
0.25 M MOPS pH 7	1.53 x 10^4	4.29 x 10^3	**=100%**	28%
0.25 M HEPES pH 7	1.36 x 10^4	5.45 x 10^3	89%	36%
0.25 M HEPPS pH 8	1.31 x 10^4	2.98 x 10^3	86%	20%
0.25 M CAPSO pH 9	4.48 x 10^3	1.94 x 10^3	29%	13%

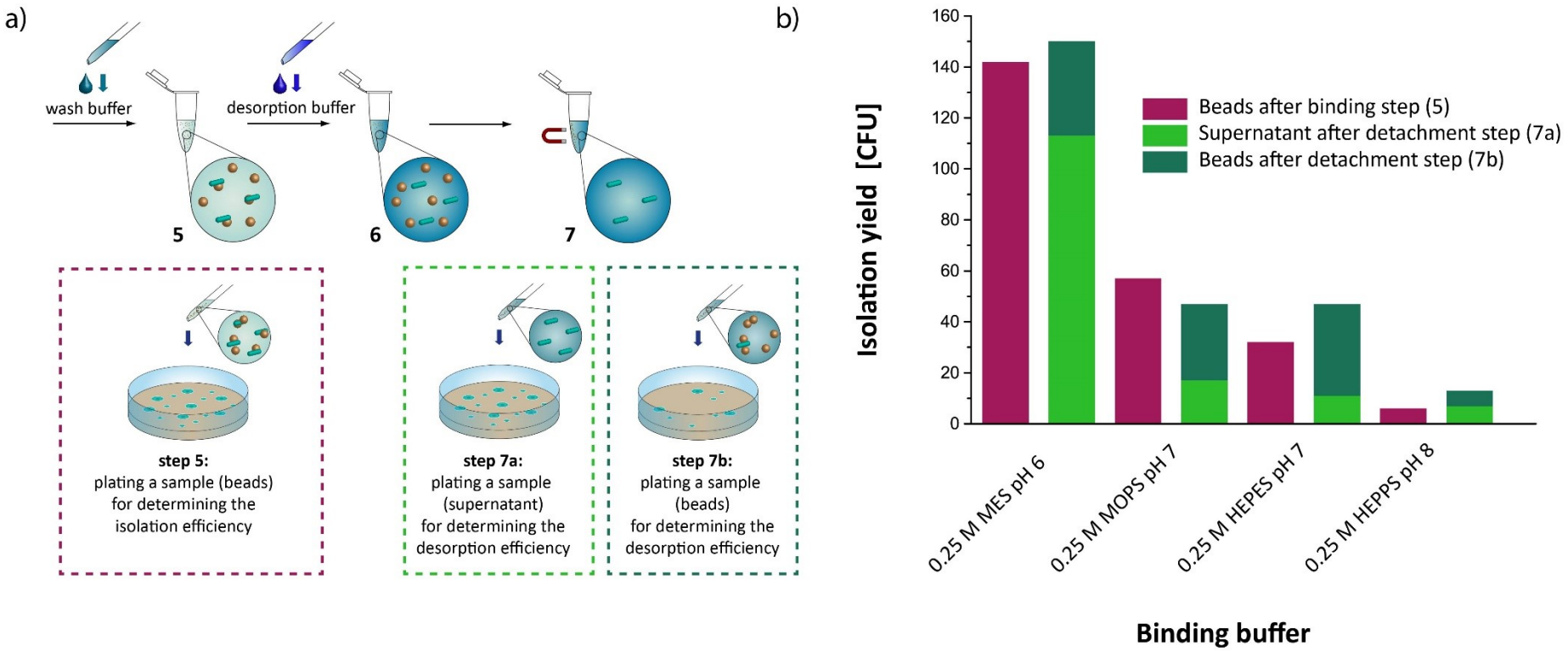

Figure S6. a) Schematic display of the plate counting method for determining the isolation yields. b) Colum diagram of one sample per binding buffer condition (therefore, no standard deviation is shown) for demonstrating that the proposed quantification method is working with reasonable precision.
The CFU numbers of the step 7a and 7b should approximately add up the CFU number of step 5.

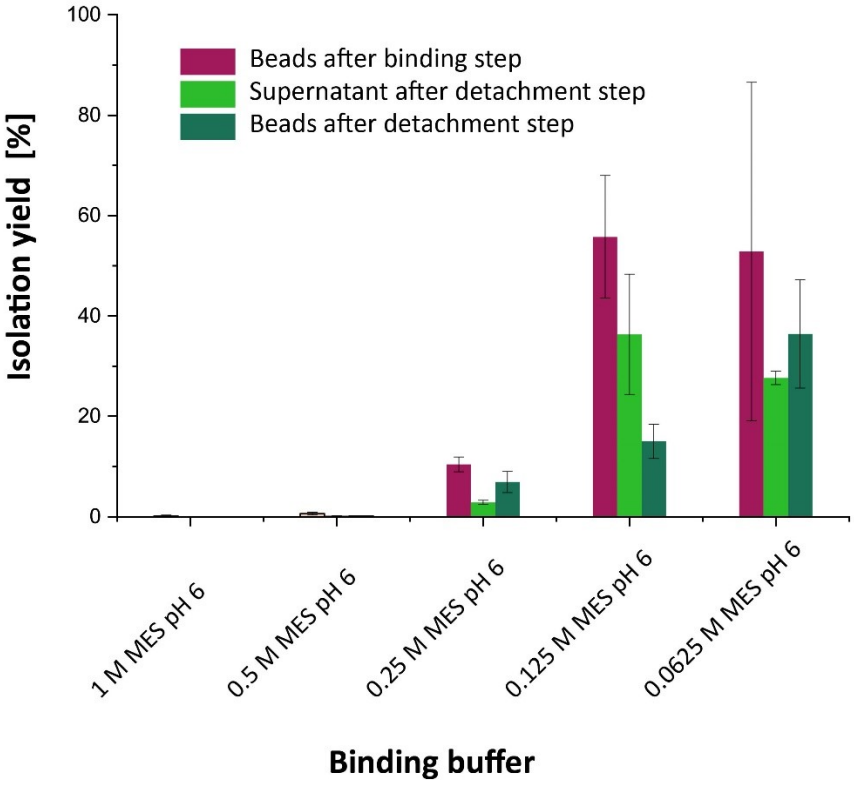

Figure S7. Influence of the binding buffer concentration on the isolation efficiency. The isolation yields were calculated by comparing the CFU counts of plated samples after different steps in the protocol to those of reference samples. To the reference samples the same number of cells (*E. coli* BL21 C+ approximately 10^3 CFU/ml) was added as initially to the processed samples. For each buffer concentration a different reference sample was used, consisting of the same buffer used in the binding step. The reference samples were incubated at room temperature for the same time that was required for the entire isolation protocol.

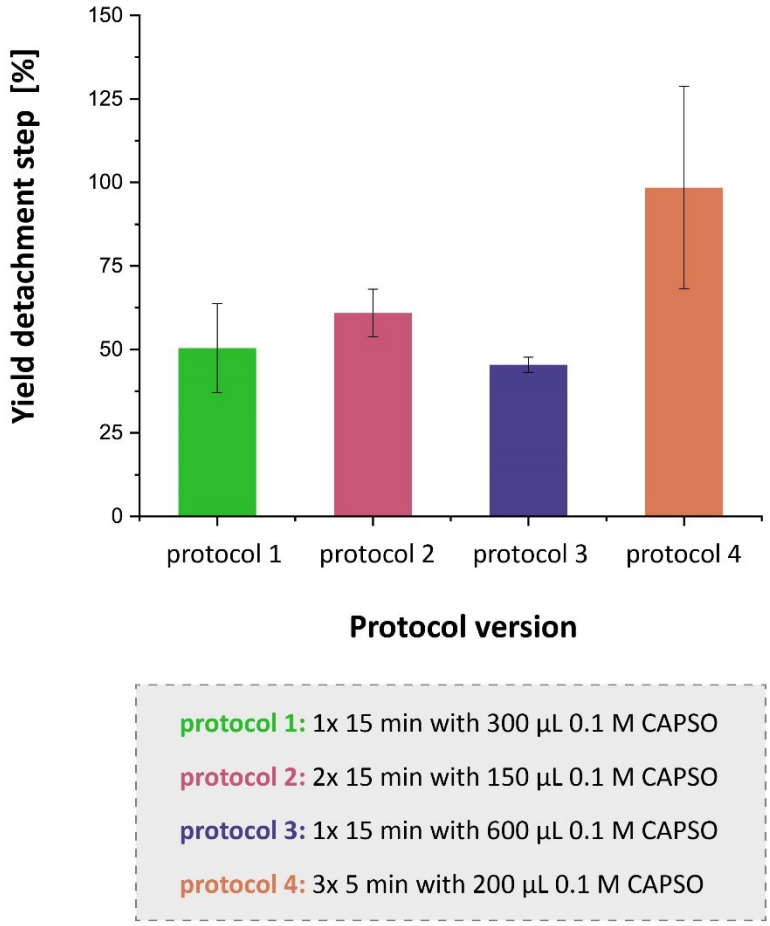

Figure S8. Optimization of the detachment of the bacterial cells from the magnetic beads. The binding step was carried out each time with 0.125 M MES buffer and the yield of the detachment step was calculated relative to the total number of cells isolated in the binding step (= 100%). For each protocol a sample with *E. coli* BL21 C+ with a concentration of approximately 10^3 CFU/ml was used.