Supplementary Material

Human deoxycytidine kinase is a valuable biocatalyst for the synthesis of nucleotide analogues

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Table S1. Phosphorylation of nucleoside analogues cladribine, fludarabine and clofarabine by *Hs*dCK. Enzymatic activity per ml of matrix was determined in reactions performed in 2 mM potassium phosphate buffer (pH 7) with 10 mM $MgCl_2$, 0.4 mM of GTP and 0.333 mM of substrate in a reaction volume of 1 ml. A reaction temperature of 37°C was used. 0.01 mg ml^{-1} of enzyme was added to the reaction. The reactions were monitored over a period of 60 min. Mean values and standard deviations were calculated for each of the three replicas of immobilization experiment 3.

Substrate	Enzyme	Enzymatic activity per ml of matrix [U ml^{-1}]
Cladribine	immobilized	1.06 (±0.07)
Fludarabine	immobilized	1.15 (±0.46)
Clofarabine	immobilized	0.76 (±0.15)

Table S2. Scale up of the synthesis of fludarabine-5′-monophosphate to 3 folds (1 mM), 19.5 folds (6.5 mM) and 36 folds (12 mM) using both free and immobilized *Hs*dCK. Product formation was studied by HPLC analysis. Formation [%] of fludarabine-5′-monophosphate and final product concentrations were determined. Formation [%] of fludarabine-5′-monophosphate was calculated from the ratio of fludarabine-5′-monophosphate concentration to the sum of concentrations of fludarabine and fludarabine-5′-monophosphate. Reactions were performed in 2 mM potassium phosphate buffer (pH 7) and 10 mM $MgCl_2$ in a volume of 100 µl and using a reaction duration of 18 h. Mean values and standard deviations were calculated from two independent experiments performed with pooled replicas of immobilization experiment 3.

Enzyme	Formation of fludarabine-5′-monophosphate [%]	Concentration of fludarabine-5′-monophosphate [mM]
soluble	71.0 ± 1.3	0.8 ± 0.04
immobilized	44.0 ± 1.8	0.5 ± 0.03
soluble	69.1 ± 1.8	5.0 ± 0.12
immobilized	37.1 ± 11.9	2.2 ± 0.8
soluble	60.2 ± 3.5	8.7 ± 0.2
immobilized	55.6 ± 7.1	6.0 ± 0.8

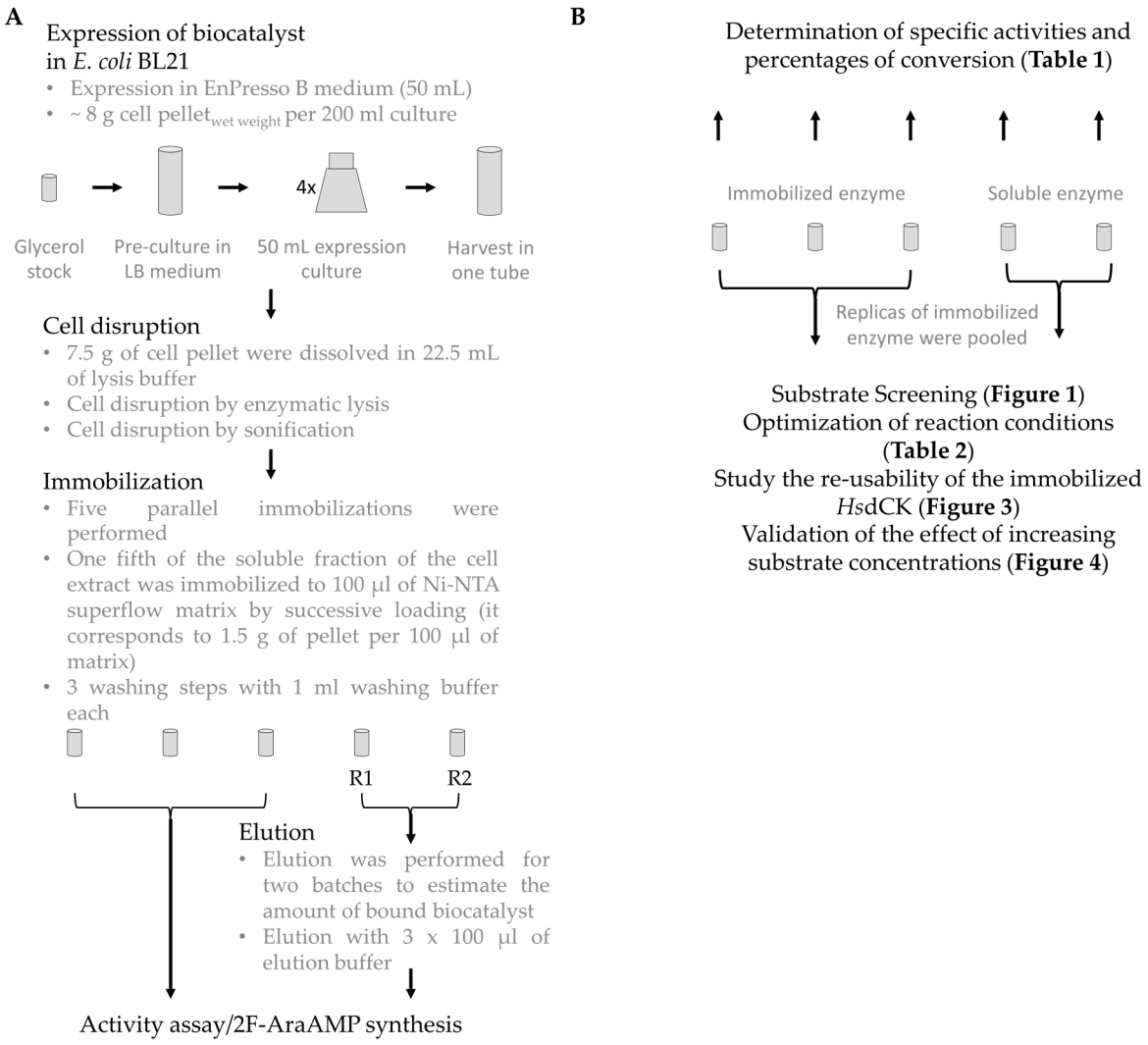

Figure S1. Methodology applied to study soluble and immobilized *Hs*dCK. (**A**) Protocol applied for one immobilization protocol. (**B**) Soluble and immobilized were either applied as separate samples or after pooling of Replicas.

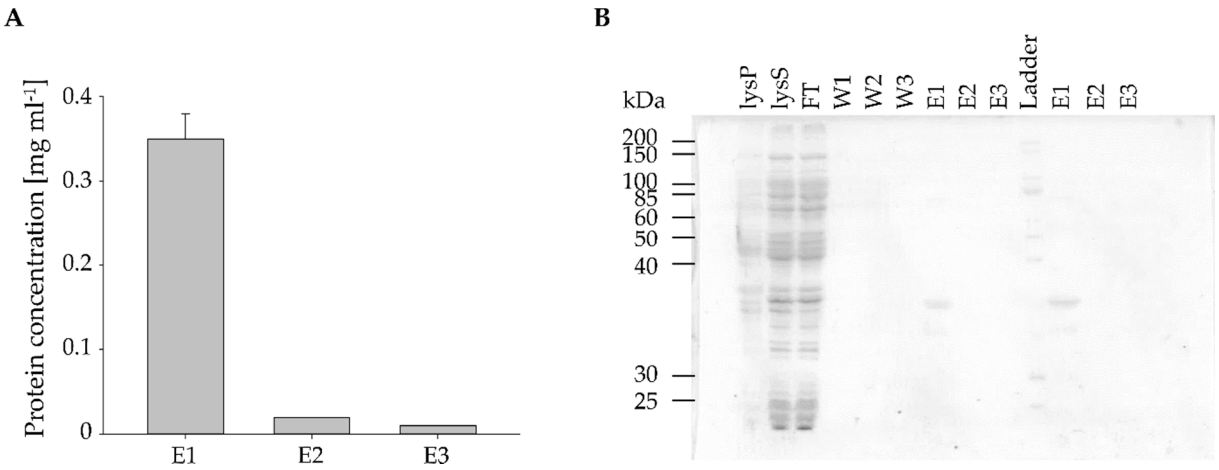

Figure S2. Purification of *Hs*dCK by Ni-NTA sepharose using 0.5 mg of pellet for 100 µl of matrix. Protein elution was performed using 500 µl of elution buffer. (**A**) Protein concentration in the elution fractions were determined by NanoDrop measurements. (**B**) Purification of *Hs*dCK was evaluated by SDS-PAGE.

lysP – Pellet after cell lysis, lysS – Soluble fraction of the crude extract, FT – flow through; W1-W3 – washing fractions; E1-E3 – elution fractions

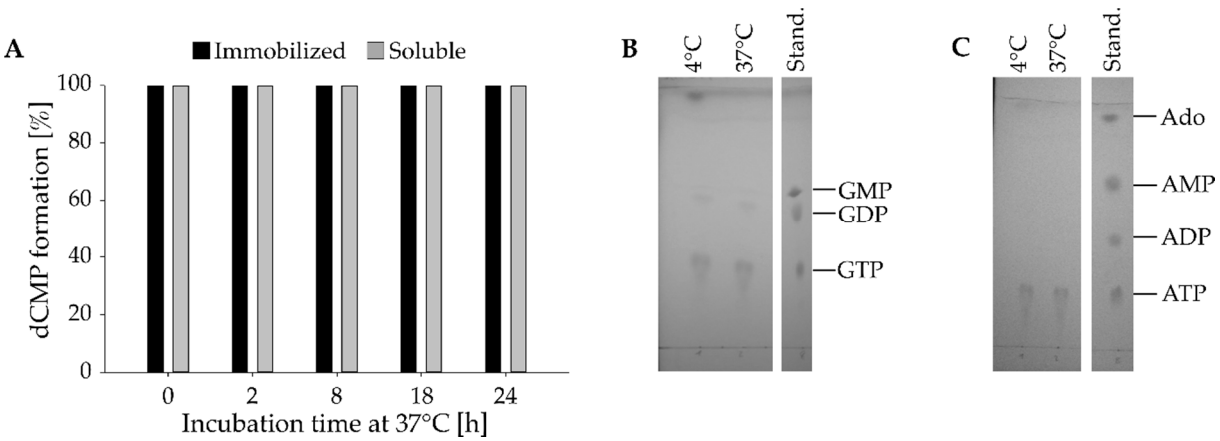

Figure S3. Stability of soluble and immobilized *Hs*dCK at 37°C (**A**) and of GTP (**B**) and ATP (**C**) at different temperatures over a period of 24 h. (**A**) Biocatalysts (0.01 mg ml^{-1}) was added to a solution containing 2 mM phosphate buffer (pH 7.0), with 10 mM $MgCl_2$ and 5 mM DTT. The mixture was stirred at 37°C for 24 h. At fixed time points, 94 µl sample were taken and supplemented with 0.4 mM of ATP and 0.333 mM deoxycytidine to start the enzymatic reaction. The enzymatic reaction was incubated with continuous horizontal shaking at 200 rpm for a period of 24 h. Control reactions were used without the addition of enzyme. No decrease in ATP concentration was observed in the negative controls. Percentage of conversion was evaluated by the photometric assay. (**B**) GTP was incubated in reaction buffer for 24 h at 4°C and 37°C. Traces of GDP were observed as impurity of the GTP stock. (**C**) ATP was incubated in reaction buffer for 24 h at 4°C and 37°C. No degradation was observed within an incubation period of 24 h. Thin-layer chromatography was performed using silica plates and dioxane, isopropanol, 25% ammonia and water (4:2:3:4) as solvents. 3.5 µl of each sample were loaded to the plates. Compounds on the silica plates were detected using UV light.

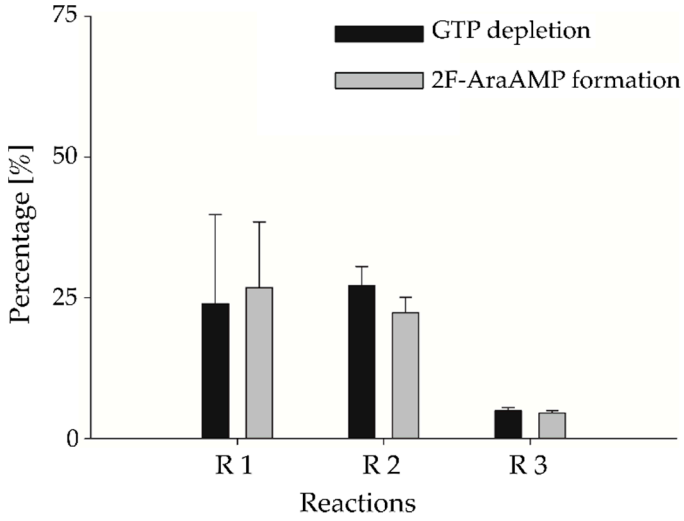

Figure S4. Reusability of the immobilized *Hs*dCK for the synthesis of fludarabine-5′-monophosphate. The formation of fludarabine-5′-monophosphate and the consumption of the phosphate donor GTP was evaluated by HPLC measurements. The enzyme was used in three consecutive reactions denoted as reaction 1, reaction 2 and reaction 3 respectively. Reactions were performed in 2 mM potassium phosphate buffer (pH 7) with 10 mM MgCl2, 0.4 mM of GTP and 0.333 mM of substrate in a reaction volume of 1 ml using a reaction time of 3 h for each reaction. Mean values and standard deviations were calculated from triplicate reactions performed with pooled replicas of immobilization experiment 3.

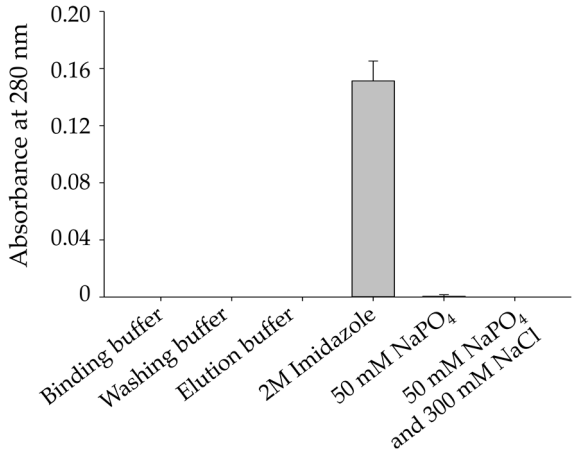

Figure S5. Determination of the absorbance at 280 nm of solutions used for the purification of *Hs*dCK using the NanoDrop. Deionized water was used as blank. Minimal absorption was only detected with the imidazole stock solution (2M imidazole).