

SI #1: Preparation of the 3', 5'-cAMP, H⁺-form

The process by the manufacturer BioLog consisted in the following: 735 μmol cAMP, Na⁺ (BioLog LSI Catalog No. B 004), MW 367.2 g/mol was dissolved in 490 mL deionized H₂O (1.5 mM) and applied to a cation exchange column pre-equilibrated to the H⁺-form (5 L 0.5 M HCl followed by 10 L H₂O until neutral) at a flow rate of 5 mL/min. The strong cation exchanger Toyopearl SP-650M (TOSOH Bioscience, Stuttgart, Germany) (binding capacity: 0.15 meq / mL corresponding to a total of 73500 meq) was used. This translated to a 100-fold excess of hydrogen over sodium, if 735 μmol cAMP, Na⁺ was applied to the cation exchanger. All product fractions with a concentration higher than 1 mM cAMP, H⁺ were pooled and stored at +4°C. The column was regenerated with 5 L 0.5 M HCl and washed with 10 L H₂O to remove all residual Na⁺ ions. Afterwards, the cAMP, H⁺ was applied again to the column to remove all remaining traces of Na⁺ ions. The resulting product fractions were pooled and filtered through a 0.2 μm membrane. The concentration was adjusted to 1 mM cAMP, H⁺ with deionized H₂O and the solution was frozen at -26°C.