

In one aspect, the present disclosure refers to a poly(N)polymerase characterized in that the poly(N)polymerase is a bacterial poly(N)polymerase and immobilized onto a solid support by covalent binding, wherein the poly(N)polymerase stays active when immobilized onto a solid support and wherein the poly(N)polymerase is selected
5 from the group consisting of poly(A)polymerase, poly(U)polymerase, poly(G)polymerase and poly(C)polymerase, wherein the poly(N)polymerase is obtained by a process comprising reacting a functional group of an amino acid residue of the poly(N)polymerase with a chemically activated solid support such that a covalent bond is formed and wherein the functional group is selected from primary amines at the N-terminus
10 of each polypeptide chain and in the side-chain of lysine, α -carboxyl groups at the C-terminus, β -carboxyl groups and γ -carboxyl groups of aspartic and glutamic acid, and sulfhydryl or thiol groups of cysteines. In another aspect, the present disclosure refers to a method for producing the poly(N)polymerase, as disclosed herein, comprising reacting a functional group of an amino acid residue of a poly(N)polymerase with a
15 chemically activated solid support such that a covalent bond is formed and wherein the functional group is selected from primary amines at the N-terminus of each polypeptide chain and in the side-chain of lysine, α -carboxyl groups at the C-terminus, β -carboxyl groups and γ -carboxyl groups of aspartic and glutamic acid, and sulfhydryl or thiol groups of cysteines. In another aspect, the present disclosure refers
20 to use of a poly(N)polymerase as disclosed herein being immobilized onto a solid support for producing polynucleotidylated ribonucleic acid (poly(N)RNA) molecules. In yet another aspect, the present disclosure refers to an enzyme reactor comprising a poly(N)polymerase being immobilized onto a solid support by covalent binding and comprising a poly(N)polymerase as disclosed herein or comprising a
25 poly(N)polymerase obtainable by the method disclosed herein. In a further aspect, the present disclosure refers to a kit comprising a poly(N)polymerase characterized in that the poly(N)polymerase is immobilized onto a solid support by covalent binding, wherein the poly(N)polymerase is the poly(N)polymerase disclosed herein, or the poly(N)polymerase obtainable by the method disclosed herein, a poly(N)polymerase
30 reaction buffer, and nucleoside monophosphates.

The immobilization of the PNP onto a solid support has a number of advantages over classical methods wherein the PNP is free in solution together with the other

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components of the polynucleotidyl reaction, such as RNA molecules, nucleotides, salts, buffer components etc.

First a PNP which is immobilized onto a solid support may be used repeatedly and

Claims

1. Poly(N)polymerase characterized in that the poly(N)polymerase is a bacterial poly(N)polymerase and immobilized onto a solid support by covalent binding, wherein the poly(N)polymerase is selected from the group consisting of poly(A)polymerase,
5 poly(U)polymerase, poly(G)polymerase and poly(C)polymerase, wherein the poly(N)polymerase is obtained by a process comprising reacting a functional group of an amino acid residue of the poly(N)polymerase with a chemically activated solid support such that a covalent bond is formed and wherein the functional group is selected from primary amines at the N-terminus of each polypeptide chain and in the side-chain of lysine, α -
10 carboxyl groups at the C-terminus, β -carboxyl groups and γ -carboxyl groups of aspartic and glutamic acid, and sulfhydryl or thiol groups of cysteines.
2. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is a poly(A)polymerase.
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3. The poly(N)polymerase according to any one of the preceding claims, wherein, the poly(N)polymerase is immobilized by covalent binding to a thiol-activated solid support, haloacetyl functionalized solid support, epoxy-functionalized solid support, pyridyl disulfide-functionalized solid support, maleimide-activated solid support or a mixture
20 thereof.
4. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase is immobilized via a thiol group of at least one cysteine residue.
- 25 5. The poly(N)polymerase according to any one of the preceding claims, wherein the covalent binding is a disulfide bridge, thioester bond or a thioether bond.
6. The poly(N)polymerase according to any one of the preceding claims, wherein the solid support comprises a member selected from the group consisting of sepharose, thiopropyl-sepharose, sephadex, agarose, silica, magnetic beads, methacrylate beads, and
30 nanoparticles, preferably the solid support comprises a member selected from the group consisting of sepharose, thiopropyl-sepharose, sephadex, agarose, silica, magnetic beads, and nanoparticles.

7. The poly(N)polymerase according to any one of the preceding claims, wherein the solid support comprises a reactive group selected from the group consisting of thiol, haloacetyl, pyridyl disulfide, epoxy, maleimide and mixtures thereof.
- 5 8. The poly(N)polymerase according to any one of the preceding claims, wherein the solid support is selected from the group consisting of activated thiol sepharose, thiopropyl-sepharose, thiol-activated sephadex, thiol-activated agarose, silica-based thiol-activated matrix, silica-based thiol-activated magnetic beads, pyridyl disulfide-functionalized nanoparticles, maleimide-activated agarose and mixtures thereof.
- 10 9. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase is derived from *Escherichia coli*, *Streptomyces coelicolor*, *Meiothermus silvanus*, *Bacillus subtilis*, *Thermus aquaticus*, *Shigella flexneri*, *Shigella dysenteriae*, *Citrobacter koseri*, *Salmonella bongori*, *Salmonella enterica*, *Trabulsiella guamensis*,
15 *Kluyvera ascorbata*, *Citrobacter freundii*, *Enterobacter cloacae*, *Enterococcus gallinarum*, *Grimontia indica*, or *Salinivibrio costicola*.
10. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase comprises an amino acid sequence being at least 80% identical to an
20 amino acid sequence as depicted in any one of SEQ ID NOs: 1 to 22, 24 to 155, or 203.
11. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase comprises at least one newly introduced cysteine residues compared to a native poly(N)polymerase, preferably the poly(N)polymerase comprises an amino acid
25 sequence as depicted in any one of SEQ ID NOs: 17-22, 32-155, or 203.
12. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase comprises only one cysteine residue or is mutated to comprise only one cysteine residue, preferably the poly(N)polymerase comprises an amino acid sequence as
30 depicted in any one of SEQ ID NOs: 2, 16-22, 24-83, 85-111, 113-139, 141-144, 146-148, 150-152, 154-155, or 203.
13. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase comprises a linker element as depicted in SEQ ID Nos: 156-180.

14. The poly(N)polymerase according to any one of the preceding claims, wherein the poly(N)polymerase comprises a purification tag as depicted in SEQ ID NOs: 181-201.
15. Method for producing the poly(N)polymerase of any one of claims 1 to 14,
5 comprising reacting a functional group of an amino acid residue of a poly(N)polymerase with a chemically activated solid support such that a covalent bond is formed and wherein the functional group is selected from primary amines at the N-terminus of each polypeptide chain and in the side-chain of lysine, α -carboxyl groups at the C-terminus, β -carboxyl groups and γ -carboxyl groups of aspartic and glutamic acid, and sulfhydryl or thiol groups of
10 cysteines.
16. Use of a poly(N)polymerase of any one of claims 1 to 14 being immobilized onto a solid support for producing polynucleotidylated ribonucleic acid (poly(N)RNA) molecules.
- 15 17. Enzyme reactor comprising a poly(N)polymerase being immobilized onto a solid support by covalent binding and comprising a poly(N)polymerase according to any one of claims 1 to 14 or comprising a poly(N)polymerase obtainable by the method according to claim 15.
- 20 18. A kit comprising
a poly(N)polymerase characterized in that the poly(N)polymerase is immobilized onto a solid support by covalent binding, wherein the poly(N)polymerase is the poly(N)polymerase according to any one of claims 1 to 14 or the poly(N)polymerase obtainable by the method according to claim 15,
25 a poly(N)polymerase reaction buffer, and
nucleoside monophosphates.