In one embodiment the chelating agent is selected from the group comprising ethylendiamine, nitrilotriacetic acid (NTA), ethylene glycol tetraacetic acid (EGTA), diethylene triamine pentaacetic acid (DTPA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), Ethylenediamine-*N,N'*-disuccinic acid (EDDS), phosphonates (e.g. Ethylenediamine tetra(methylene phosphonic acid) EDTMP or Diethylenetriamine penta(methylene phosphonic acid) DTPMP), citrate, porphyrines (e.g. heme, chlorophyll or vitamin B12). In one embodiment the chelating agent is ethylenediaminetetraacetic acid (EDTA).

There is also provided a method for producing a polypeptide comprising the step of incubating (resuspended) prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 6mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C. In one aspect, there is provided a method for producing a recombinantly produced polypeptide comprising incubating resuspended prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 6mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C, wherein the solution is substantially free of peptidoglycan hydrolyzing enzymes and sugar; and isolating the recombinantly produced polypeptide from the periplasm of the prokaryotic cells.

There is also provided a method for producing a polypeptide comprising the step of incubating (resuspended) prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 4mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C.

In one embodiment the concentration of the chelating agent is from about 1mM to about 6mM.In one embodiment the concentration of the chelating agent is from about 1mM to about 4mM. In one embodiment the concentration of the chelating agent is from about 1mM to about 3mM. In one embodiment the concentration of the chelating agent is from about 2mM to about 6mM. In one embodiment the concentration of the chelating agent is from about 2mM to about 4mM. In one embodiment the concentration of the chelating agent is about 2mM. In one embodiment the concentration of the chelating agent is about 4mM. In one embodiment the concentration of the chelating agent is about 6mM.

In one embodiment the solution used in the method as reported herein is substantially free of sugar. In one embodiment the sugar is sucrose.

In one embodiment the solution used in the method as reported herein is substantially free of sugar and substantially free of peptidoglycan hydrolyzing enzymes. In one embodiment the solution used in the method as reported herein is substantially free of sucrose and substantially free of lysozyme.

In one embodiment the method further comprises after the incubating step the step of isolating the polypeptide.

In one embodiment the method comprises the step of purifying the isolated polypeptide.

In one embodiment the method is characterized in that the EDTA concentration is about 2mM, the pH value is about 8, the incubation time is about 30min, the concentration of Tris-HCl is about 20mM, the incubation temperature is about 25°C and the prokaryotic cell is an E.coli cell.

In one embodiment the method is characterized in that the EDTA concentration is about 4mM, the pH value is about 8, the incubation time is about 30min, the concentration of Tris-HCl is about 40mM, the incubation temperature is about 25°C and the prokaryotic cell is an E.coli cell.

In one embodiment the method is characterized in that the EDTA concentration is about 6mM, the pH value is about 8, the incubation time is about 30min, the concentration of Tris-HCl is about 60mM, the incubation temperature is about 25°C and the prokaryotic cell is an E.coli cell. One aspect as reported herein is the use of a method as reported herein for the isolation of a periplasmic polypeptide from a prokaryotic cell.

In another aspect, there is provided use of a method as defined herein for the isolation of a recombinantly produced periplasmic polypeptide from prokaryotic cells.

## Patent Claims

1. Method for producing a recombinantly produced polypeptide comprising

   - incubating resuspended prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 6mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C

   wherein the solution is substantially free of peptidoglycan hydrolyzing enzymes and sugar; and

   - isolating the recombinantly produced polypeptide from the periplasm of the prokaryotic cells.

2. Method according to anyone of the preceding claims characterized in that the pH value is about 8.

3. Method according to any of the preceding claims characterized in that the incubation time is about 30min.

4. Method according to any of the preceding claims characterized in that the concentration of Tris-HCl is about 20mM.

5. Method according to any of the preceding claims characterized in that the prokaryotic cell is a gram-negative cell.

6. Method according to any of the preceding claims characterized in that the prokaryotic cell is an E.coli cell.

7. Use of a method according to anyone of the preceding claims for the isolation of a recombinantly produced periplasmic polypeptide from prokaryotic cells.