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 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.

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.

Patent Claims

1. Method for producing a 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.

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 periplasmic polypeptide from prokaryotic cells.