Application Note No. 451, Appendix 1: Supplementary Details on Materials and Methods

Materials

Reagents

SRAM Medium:

> 100 mM MOPS buffer, pH 7.4 with 24 g/L yeast extract,16 g/L tryptone, 10 g/L casein hydrolysate and 10 mL/L glycerol (100 %)
> Autoclaving
> Addition of 500 µL/L Antifoam 204 10 % and 2.5 mL/L Kanamycin solution (10 mg/mL in 0.9 % NaCl).

1 mM IPTG solution (final concentration)

Lysis buffer:

> 0.2 g/L lysozyme solution, 3000 U/L Benzonase® nuclease, 1mM $MgCl_2$, 20 tablets/L cOmplete™, EDTA-free protease inhibitor cocktail in binding buffer

Binding buffer:

> 100 mM NaCl, 25 mM HEPES, 10 mM Imidazole in molecular biology grade water
> Adjustment of pH to 7.4
> Filtered through 0.22 µM filter before use

Elution buffers (100 % and 20 % Imidazole):

> 100 mM NaCl, 25 mM HEPES, 250 mM Imidazole (100 %) or 50 mM Imidazole (20 %) in molecular biology grade water
> Adjustment of pH to 7.4
> Filtered through 0.22 µM filter before use

Consumables

> Ultra Yield® Flasks 250 mL and 2.5 L, Sterile (Thomson)
> AirOtop™ Enhanced Seals for Ultra Yield® 250 mL Flask, Sterile, Patented (Thomson) and 2.5 L Flask, Sterile, Patented (Thomson)
> HisTrap® FF crude columns, 5 mL (GE Healthcare)

Equipment

> Pump Watson Marlow 323 dz
> Q500 Sonicator with ½" probe (QSonica®)
> NanoDrop® ND 2000 Spectrophotometer (Thermo Fisher Scientific)
> xMark Microplate Spectrophotometer (BioRad®)

Methods

Photometric analysis

Photometric total protein quantification by absorbance measurement at 280 nm.

SDS PAGE electrophoresis and Coomassie Blue® staining

The purity of the protein hPSP and the presence of contaminants in the different fractions were verified by 4-12% SDS-PAGE stained with Coomassie Blue. Sample and buffer preparation for gel electrophoresis in reducing and denaturation conditions were prepared following the NuPAGE® technical Guide (Thermo). 4.37 µl of NuPAGE LDS sample buffer and 1.75 µl of NuPAGE Sample Reducing Agent have been added to a volume of 11.4 ul of sample from each fraction. The mixture was heated at 70°C for 10 minutes to complete the reducing and denaturation conditions before loading each sample (16 µl) into the wells of the NuPAGE 4-12% bis-Tris Protein gel, 1.0 mm, 10-well (Thermo). Protein molecular weight marker has been loaded into each gel. The gel was run in XCell SureLock® Mini-Cell (Thermo) and NuPAGE MES SDS Running Buffer (Thermo) at 200 V constant, current: start: 125 mA/gel, end: 70-80 mA/gel for 35 minutes. After electrophoresis the gel was washed with deionized water for 15 minutes under agitation at 60 rpm on an orbital shaker. The stain process was proceeded using the reagent GelCode® Blue Safe protein stain (Thermo) under agitation at 60 rpm for 1 hour at room temperature. Staining reagent was replaced with ultrapure water and gel was destained for 2 hours at 60 rpm and room temperature before scanning.

Enzyme activity assay

The activity was assayed by the determination of phosphate using the Malachite green phosphate assay kit (Sigma). The method relies on the use of malachite green to quantify the amount of phosphomolybdate formed at 620 nm in presence of inorganic phosphate released by the enzyme. 80 µl of each sample were placed into a 96 well plate.
20 µL of working reagent was added into each well. The samples were incubated 30 minutes at room temperature for color development. Afterwards the absorbance was measured at 620 nm on a plate reader.

Find here the related application note
https://eppendorf.group/jh512a

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