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Western Blot CTB

PLOS Neglected Tropical Diseases

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1 Works for me dx.doi.org/10.17504/protocols.io.bastieen

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ABSTRACT

For studying intracellular accumulation of CTB subunits in *E coli*, bacteria were grown up to 4 hours following induction by 0.2% of arabinose. Post induction, bacterial cultures were mixed with equal volume of translation-translocation halt cocktail (200 µg/ml chloramphenicol, 200 mM sodium azide, 9.5% ethanol) and immediately placed in an ice water bath for 15 minutes of incubation to arrest protein synthesis and translocation. Cells were harvested by centrifugation at 14000 rpm for 15 minutes at 4°C. Supernatants were preserved and used for CT-ELISA experiment as described before. Pellets were then suspended in TME buffer (20 mM Tris-Cl pH 8.0, 2mM β-mercaptoethanol, 1 mM EDTA), preferably $1/10^{th}$ volume of the original culture and disrupted by sonication. After centrifugation at 13,000 rpm at 4°C for 15 min to remove any unbroken cells, crude cell lysates were used for measurement of protein concentration using Bradford assay (BioRad, USA). Equal amount (≥80 µg) of protein samples were mounted on to 15% SDS-PAGE. Blots were probed with classical CTB-specific monoclonal antibody (anti-Cla CTB, 1:5000) or anti-beta lactamase (anti-βla 1:500) antibody. A portion of the same blot was stained with Ponceau S staining solution (Sigma-Aldrich, St Louis, MO, USA) to validate whether equal amount protein samples were transferred in the blot. Blots were developed using West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL) according to the manufacturer's instructions and visualized in ChemiDoc XRS$^+$ system (BioRad, USA). Densitometric analysis of band intensities was performed using the Multi Gauge software V2.3(FujiFilm).

EXTERNAL LINK

https://doi.org/10.1371/journal.pntd.0008128

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Ryan JP, Bassford Jr PJ. Post-translational export of maltose-binding protein in Escherichia coli strains harboring malE signal sequence mutations and either prl+ or prl suppressor alleles. J. Biol. Chem. 1985; 260: 14832-14837.

ATTACHMENTS

Protocol 1_WB CTB.docx

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