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## Localization of Leptospiral surface proteins by biotinylation

This is a biotinylation protocol specifically developed for assessing surface-exposure of leptospiral proteins. Viable, intact spirochetes are labeled with the water-soluble, membrane-impermeable reagent, Sulfo-succinimidyl-6-(biotinamido) hexanoate (Sulfo-NHS-LC-Biotin). Affinity capture of biotinylated proteins from intact cells by streptavidin allows to identify all surface-exposed proteins. Proteins are blotted to PVDF membrane and the biotin labeled proteins detected by streptavidin horseradish peroxidase (HRP) conjugate (biotin ligand blot). The localization of a protein of interest can be done by assessing biotinylation products from intact and lysed cells using immunoblotting. This technique requires employment of several negative controls (immunoblot with antibodies for subsurface, preferably periplasmic proteins) to assess the integrity of the outer membrane during biotinylation labeling of intact cells.

### Method:

1. Grow *Leptospira* in EMJH medium, supplemented with 1% rabbit serum at 30° C until they reach density of  $\sim 5 \times 10^8$  cells/ml.
2. Harvest *Leptospira* culture by low-speed centrifugation at 2,000 x g for 7 min at room temperature
3. Gently resuspend cells in PBS containing 0.4 mg/ml sulfo-succinimidyl-6-(biotinamido) hexanoate (Sulfo-NHS-LC-Biotin) to a final concentration of  $2 \times 10^9$  cells/ml.
4. Incubate the reaction for 1 min and quench residual Sulfo-NHS-LC-Biotin by addition of 50 mM Tris-HCl (pH 8.0) for 5 min at room temperature.
5. Remove inactivated Sulfo-NHS-LC-Biotin by two washes in PBS.
6. As labeling control, prepare Sulfo-NHS-LC-Biotin labeled lysates:
  - lyse cells by three rounds of freezing (10 min at -80° C) and thawing (15 min at room temperature)
  - label the lysates as described above.
7. Extract labeled proteins:
  - resuspend  $1 \times 10^9$  bacteria in 500  $\mu$ l of 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 2 mM EDTA, 0.2% SDS and boil for 5 min.
  - Affinity-capture biotinylated proteins with EZview Red Streptavidin Affinity gel (Sigma-Aldrich) according to manufacturer's instructions.
8. Analyze the biotinylated surface proteins and biotinylated controls (cell lysates) by SDS-PAGE and biotin ligand blotting or immunoblotting.

### References:

1. Cullen, P., Xu, X., Matsunaga, J., Sanchez, Y., Mastali, M., Ko, A. I., Haake, D. A., and Adler, B. "The Surfaceome of *Leptospira*" *Infect. Immun.* 73:4853-63 (2005).
2. Pinne, M. and Haake, D. A. "A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of *Leptospira interrogans*," *PLoS ONE* 4:e6071 (2009). PMID: 19562037.