NLP screening (dot blot)

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ABSTRACT

In order to determine if the His-tagged NLP is expressed and secreted by the bacteria, we plan to use the dot blot procedure with anti-His tag anti-bodies. Dot blot is an immunological technique used for detecting proteins directly from the culture supernatant (without gel separation). Thus, the samples are directly spotted on the membrane, making it a high throughput procedure ideal for testing different secretion signals.

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PROTOCOL

MATERIALS TEXT

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- TBS: 20 mM Tris-HCl 150 mM NaCl pH 7.5
- TBS-T: 0.05% Tween20 in TBS
- BSA/TBS-T: 0.1% BSA in TBS-T

1 On nitrocellulose membrane indicate the blotting region by drawing a grid by pencil.

2 Slowly apply 2 µl of sample on the nitrocellulose membrane at the center of the grid.

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3. Use purified NLP-His and apply 5 points of increasing concentration of them on the nitrocellulose membrane in order to create a standard concentration curve.

4. Dry the membrane.

5. Block non-specific binding sites by washing the membrane in 5% BSA in TBS-T for 1h at room temperature.

6. Incubate with primary antibody diluted at the concentration recommended by the producer for 30 min. This step may require optimizing of the concentration.

7. Wash 3 times with TBS-T for **00:05:00**

8. Incubate with the secondary antibody conjugated with HRP for **00:30:00** (concentration recommended by the producer).

9. Wash 3 times with TBS-T (15 min, 5 min, 5 min)

10. Wash with TBS

11. Incubate with ECL reagent for 1 min. Develop the blot.

12. Compare the intensity of the sample with the standard curve in order to estimate the concentration.