CFSE LABELING OF *STREPTOCOCCUS PNEUMONIAE*

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BACKGROUND

For assays where it is important to track bacteria (ie subcellular location) fluorescent dyes can be used. Many of these dye intercalate the bacterial membrane or reside in the cytoplasm; for the purpose of this protocol the dye Carboxyfluorescein succinimidyl ester (CFSE) is employed. CFSE is a good alternative to FITC as it is much brighter, however this can also cause considerable spillover into other channels.

NOTES

- The most critical aspect of this protocol is that bacteria are washed at least three times following labeling. Residual CFSE in solution will non-specifically label host cells causing increased background noise.
- OD600 absorbance is a convenient way to approximate bacterial concentration, however plating serial dilutions of bacteria should be used for an accurate quantification.

EQUIPMENT

- CFSE dye (Sigma #21888)
- Nutator mixer or equivalent mixer/shaker
- 1X PBS
- *Streptococcus pneumoniae* in TSB broth

PROTOCOL

1) Grow *S. pneumoniae* to approximately 0.5 OD600 in a 5 ml TSB culture at 37°C.

2) Centrifuge culture at 4000g for 5 mins, and fully resuspend pellet with 2 ml of 10 µM CFSE in PBS.

3) Incubate at room temperature for 1 hr, in the dark, while gently rocking or shaking to ensure even staining.

4) Centrifuge culture at 4000g for 5 mins, and resuspend pellet in 15ml PBS. Repeat twice.

5) After final wash, fully resuspend pellet with 5ml PBS. Alternatively, resuspend pellet in 1-2 ml PBS, and adjust culture to desired OD600 absorbance.

6) To heat kill bacteria, incubate at 65°C for 10mins and store at 4°C for later use.