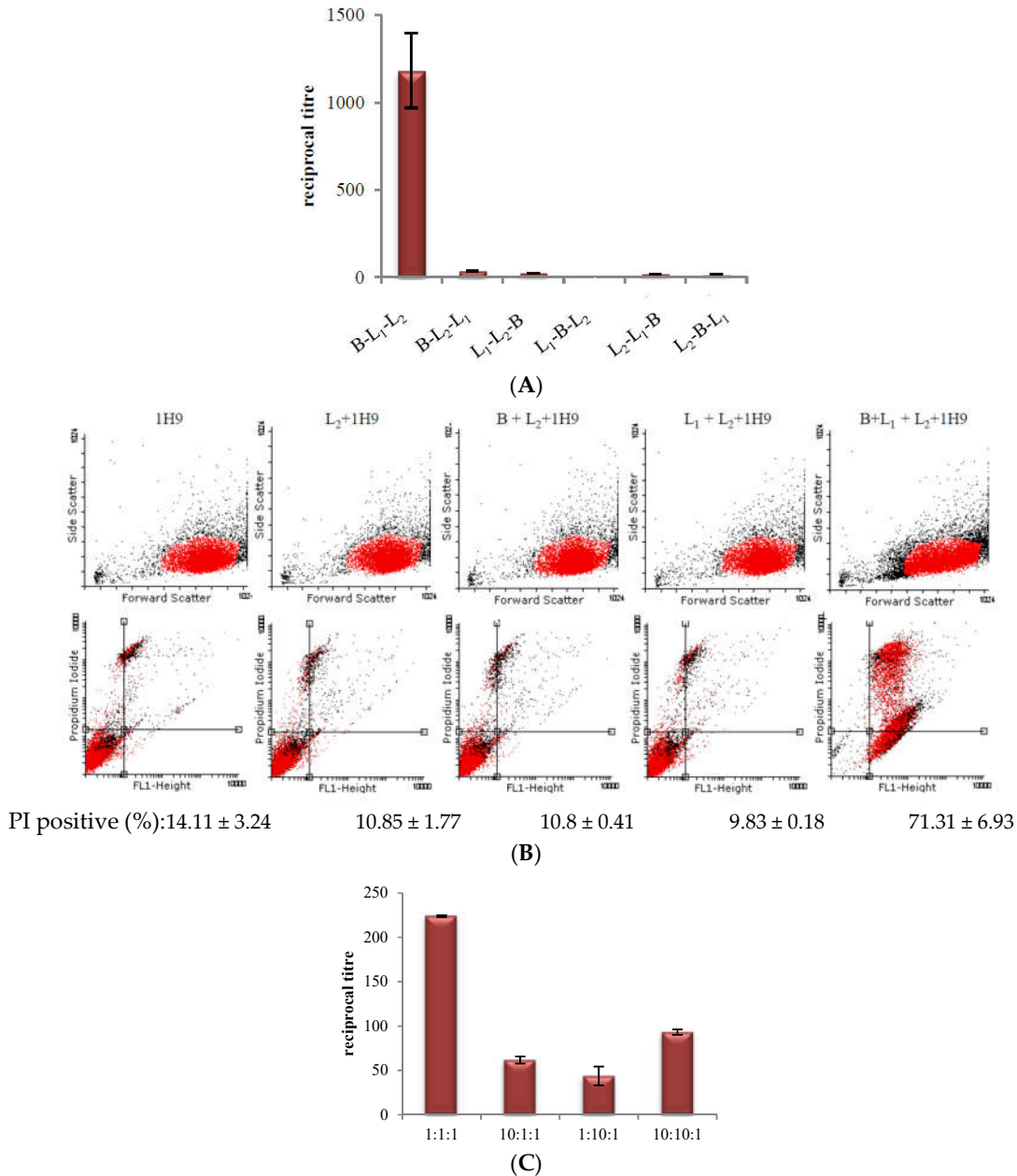


# Supplementary Materials: Binding to The Target Cell Surface is The Crucial Step in Pore Formation of Hemolysin BL from *Bacillus cereus*

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**Figure S1.** Additional results on cytotoxicity and cell binding of *B. cereus* Hbl. **(A)** WST-1 bioassay. rHbl components were applied to Vero cells consecutively with two intermediate washing steps. rHbl B and L<sub>1</sub> were applied constantly (3.75 pmol/mL), L<sub>2</sub> as serial dilution, each for 1 h. After the third component, cells were again washed two times and incubated with WST-1 for 1.5 h. titers were determined as the toxin dilution causing 50% dead cells. **(B)** Flow cytometry on Vero cells. Cells were incubated with different combinations of rHbl B, L<sub>1</sub>, L<sub>2</sub>, and mAb 1H9 as well as 5 µg/mL PI and Alexa Fluor® 488 goat anti mouse IgG for detection. Upper row: cell size and granularity. Lower

row: FL1 (488 nm, see Table 1) and propidium iodide (PI) signals. (C) rHbl components were applied in different concentration ratios as dilution series to Vero cells in WST-1 bioassays. 1:1:1 = 75 pmol/mL each as start dilution. 10:1:1 = 10× excess of rHbl L2. 1:10:1 = 10× excess of rHbl L1. 10:10:1 = 10× depletion of rHbl B.