mycobacterial cell wall fragments, was further purified by centrifugation washing at a higher relative centrifugal force, in order to remove soluble contaminants. The MpRNC fraction was isolated as a pellet following centrifugation washing at high relative centrifugal force. The number of live mycobacteria in the MpRNC fraction was determined by plating serial dilutions of the mycobacterial intact cell-free fraction on tryptic soy agar growth media, incubating for 72-96 h at 37°C, and determining the number of colony forming units (CFUs). The MpRNC suspension was then heat-treated at 121°C for between 5-30 min. Table 6 shows the steps used to prepare MpRNC with a high-, an intermediate-, or with a low intact mycobacterial cell content (hereafter referred to as MpRNC High, MpRNC Intermediate and MpRNC Low, respectively).

Table 6. Preparation of MpRNC containing High, Intermediate and Low levels of intact *Mycobacterium phlei*

Step	MpRNC High	MpRNC Intermediate	MpRNC Low
1	High-pressure homogenization, 10,000 PSI, 2 cycles	High-pressure homogenization, 18,000 PSI, 5 cycles	High-pressure homogenization, 27,000 PSI, 5 cycles
2	High-speed centrifugation, 38,400 x RCF, to pellet MpRNC and any remaining intact cells	Low-speed centrifugation, 3,160 x RCF to remove intact cells	Low-speed centrifugation, 3,160 x RCF to remove intact cells
3	High-pressure homogenization, 1018,000 psi, of resuspended MpRNC pellet 2 cycles	High-speed centrifugation, 38,400 x RCF to pellet MpRNC and any remaining intact cells	High-pressure homogenization, 27,000 psi, to further reduce intact cell content 3 cycles
4	MpRNC High	High-pressure homogenization, 18,000 psi, of resuspended MpRNC pellet 2 cycles	High-speed centrifugation, 38,400 x RCF to pellet MpRNC and any remaining intact cells