Supplementary material

Fig. S1. *Mycobacterium smegmatis* mc^2155 glycolipids analysis by analytical TLC staining with Coomassie blue. (A) chloroform/methanol/ammonium hydroxide (80:20:2, by vol.) mixture as mobile phase: *Mycobacterium bovis* TDM as standard (GERBU Biotechnik) (lane 1), total lipid phase containing major glycolipid (lane 2), purified TDM (lane 3) and purified TMM (lane 4). (B) chloroform/methanol (9:1 by vol.) mixture as mobile phase: total lipid phase containing major glycolipid (lane 1), purified TDM (lane 2) and purified TMM (lane 3) mycolylmannosylphosphorylheptaprenol (Myc-PL) is indicated to the right and left of the plates.

Fig. S2. SDS-PAGE (A) and immunoblotting (B) analyses of the purified recombinant antigen 85A .(A) (M): molecular weight marker (Fermentas); Lanes 35-39 represent the fraction numbers after the final purification step by size exclusion chromatography. (B) (M): molecular weight marker (Fermentas); lanes 36-40 corresponding to the fraction numbers.

Fig. S3. Influence of organic solvents on mycolyltransferase reaction. Investigation of the effect of methanol and mixture of chloroform/methanol on the reaction rate with TMM in standard buffer conditions and negative control with TDM in methanol and mixture of chloroform/methanol, respectively. Me, methanol; Cl, chloroform; Sb, standard buffer.

Tables

Table S1. Determination of Z′ factor from 96-well plate format assays at different volumes. The reaction was carried out with the indicated amount of protein in the reaction mixture, which is described in the materials and methods. The results are an average of 11 experiments of separate negative and positive control.

Reaction volume	**200 µl**	**300 µl**	**350 µl**
Z′ factor	0.67 ±0.021	0.72 ± 0.014	0.73 ±0.012

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