

Purification of artificial metabolosome proteins

The proteins of the artificial metabolosomes were purified following the procedure that had previously been described involving a series of differential spins and ultracentrifugation steps (Havemann and Bobik, 2003).

BL21(DE3) was grown in 200 ml of Luria-Bertani medium until OD₆₀₀ of 0.8 when protein production was induced with 1 mM IPTG at 16 ° C overnight.

The cells were harvested by centrifugation at 4,000 x g at 4 ° C for 10 minutes, resuspended in 10 ml sonication buffer(TEM)* and lysed by sonication (six 30 seconds bursts with 30 seconds cooling intervals on ice at an amplitude of 65%).

Subsequently, insoluble matter was removed by centrifugation at 12,000 x g for 20 min,

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The soluble clarified sample was subjected to high-speed centrifugation at 48,000 x g for 90 minutes (Beckman Optima LE-80K, SW-28 rotor).

- Recipient for TEM:
 - 50 mM Tris-HCl,
 - 2 mM EDTA,
 - 0.2% 1,2-PD [pH 8.0]

ref: Protein content of polyhedral organelles involved in coenzyme B12-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. *J Bacteriol.* 2003 Sep; **185**(17):5086-95