Lysin purification protocol

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Abstract

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Guidelines

This protocol assumes that salt extracts lysin from the virion rather than from cell walls. Yield seems to confirm this assumption.

Quality control: lysin assay shows that all chlorophyll has been released and no cell wall material pellets at low speed (wall has been solubilized).

The final high speed pellet (after step 8) showed a white layer (virus) remaining in the pellet.

Protocol

Step 1.
Pellet 500ml cells (5min x 5000RPM).

DURATION
00:05:00

Step 2.
Resuspend in 20ml MBBM, add 20µl tetracyline.

Step 3.
Add 150µl purified PBCV incubate 16hrs under fluorescent illumination with a 16 hr light/8 hr dark cycle at 25°C on an orbital shaker (95 rpm).

DURATION
16:00:00

Step 4.
Collect cells and virus by centrifugation at 25,000RPM (50.2Ti) for 17 min at 20°C.

DURATION
00:17:00

Step 5.
Re-suspend cells in 20ml 25mM KOH, 50mM MOPS, 2mM Na_2EGTA, 0.1M LiCl(pH 7) (wash).

Step 6.
Centrifuge 25,000RPM for 17 min at 20°C, discard supernatant.

DURATION
00:17:00

Step 7.
Suspend pellet in 1.5ml 25mM KOH, 50mM MOPS (pH 7), 60µl 10M LiCl, 30µl 0.1M Na_2EGTA incubate 50 min at 37°C.

DURATION
00:50:00

Step 8.
Centrifuge 25,000RPM for 17 min at 20°C. Collect supernatant (avoid DNA).

DURATION
00:17:00