

9/19/56

Purification of Polymerase

Preparation of Strip Ppt.

To 300 ml of 10' sonicate (20 mg protein/ml - bovine serum albumin as standard) add with stirring 300 ml of $\frac{1}{20}$ Tris, 7.5, add slowly with mechanical stirring 45 ml of 5% Strip Stk. Stand 10' in ice, centrifuge in 16 Serwall tubes, 5' at #85, collect ppt. (save 5.0 ml of supernatant for assay).

To each tube add 10 ml of $\frac{1}{20}$ KPO₄ 7.4 disperse ppt. with plastic homogenizer attached to stirring motor.

Note: Ppt. suspended well once, then resuspended twice more, whole operation required about 25'.

Pool suspension from all tubes, wash tubes + make up to 310 ml with $\frac{1}{20}$ KPO₄ Mix.

Spinco Treatment

Transfer suspension to Spinco Tubes (38 ml), centrifuge 120' at 30000 RPM in Spinco in Rotor 30 - Collect supernatant.

Note: There are two deviations from normal procedure here.

- 1.) Usually after suspension of Strip ppt., is centrifuged in Serwall before Spinning.
- 2.) Spinco centrifugation usually at 40000 RPM in Rotor 40 which gives higher force field.

One tube broken in centrifuge - Vol. of supernatant 237 ml.

Nuclease Treatment:

Remove 2.0 ml for Assay + Protein Detn, + UV.
To remainder ~ 235 ml make following additions:

3.7 ml of .3M MgCl₂ Worthington 1X reagent.

.23 ml of ONase " 100x/ml

.023 ml of ANAase, 1 mg/ml - Worthington

Inc 5 hrs at 37°

UV of Spinco Sup. = .02 → 1.0 at 260mμ = .278 = 13.9 OU/ml.

Nuclease Treated Spinco Sup. 0.5ml + 0.5ml of 7% PCA (old) stand

5 min in ice, spin. UV of .05 → 1.0 at 260mμ = .530 = 21.2 OU/ml ~ 50% of original

(see over)

Dialysis:

Dialyze at 2° vs 4 liters of $\frac{1}{100}$ Tris, 7.5 for 16 hrs. Moderate ppt. forms which was removed by centrifugation. ~~Concn~~
Shell freeze + concentrate by lyophilization to volume less than 30 ml. ^{show,} Transfer to cylinder + dilute to total volume of 38.0 ml with ice-water. This is "Lyophilized" "P" Enzyme