F 10 1956

Purified Pancreatic Lipase

This vial contains 5 ml. lipase prepared as follows:

Prepn:

1. 10 gm. NBC steapsin suspended vigorously in 650 ml. 10% NaCl. [The entire procedure carried out in the cold.] Spun. Use suptt.
2. Suptt brought to saturation with $MgSO_4$. Spun. Use ppt.
3. $MgSO_4$ ppt. taken up in 10% NaCl*, and dialyzed extensively (2-3 days) against distilled water.
4. After dialysis, a precipitate is present in the dialysis bag. ~~Both~~ Both the ppt. and the supt. are active, each containing about half the activity. The ppt. is taken up in 10% NaCl.*
5. Ppt. and suptt. are both kept frozen; supt goes bad within 3 mos., or sometimes much sooner; pellet retains appreciable activity after 3 mos.

This sample is from a 100-ml. batch of the pellet fraction, prepared as above 7/18/56.

Use:

This material is quite likely not to survive outside temp. long enough for mailing. If it retains its present activity, however, it is effective on *B. meg.* protoplasts at 2 ml/100 ml of protoplasts resulting from cells originally at 300 K.U. (660 mμ) or about O.D. 0.6.

Calcium is required at 10^{-2} M.

There is a suggestion of reduced activity with strong aeration; shaking during incubation should probably be slightly inadequate compared to optimal growth conditions. Effect of glutathione has not been tested.

An overnight (ca. 14 hr.) culture of smooth *B. meg.* on 2% peptone is diluted to OD 0.14 in peptone, grown 50 min. at 30° to OD 0.18 (660 mμ), washed with 0.2 M $NaPO_4$ or Na Succinate pH 7.7, and resusp. to OD 0.6 in 0.5 M Na succinate + 0.25% glutamate + $Mg\ 10^{-3}$ M + $Mn\ 10^{-4}$ M, pH 7.7. Add $CaCl_2$ 10^{-2} M, lysozyme 0.3 mg/ml, and lipase 2 ml/100 ml. Shake at 30°. Protoplasts appear at 45-60 min., nuclei at 75-120 min.

* Arbitrary volumes used were from 2-3 to 1 ml. at $MgSO_4$ step, 1-300 at end.