

SU 40 prep.

BSC-1 or CV-1 or 1° Monkey kidney
(Some claim higher yield = 1 MK, but expensive)

BSC-1 develop resistance \in \uparrow passage.

Earnest uses oldest stock, grows + freezes most of it.

From Aabin. Will test Flow lab. line.

Can get ATCC line.

Passage at high cell dens. in ear. EM \in 10% calf ser.
— can use basal EM. (1 \rightarrow 5 in passage)

3 stocks in culture + then go back to freezer

100 large plates, ^{needed} \bar{c} 10^6 cells/plate

6 days growth or just \bar{c} confluence.

Remove medium + repl. \in fresh med. cont. 2%
calf ser + stock virus (3×10^8) dil 1:1000.

n.c. 3×10^5 ^{dil.} in maint. med. n.c. moi = $\approx 1/20$

Can use moi $1/10,000$ (Yoshiiki)

Imp. to use very low multiple. to avoid defectives.

Maintain cultures until full CPE (fr. 7d. —
complete in $\approx 10-12$ d. dep on moi). Controls can
do well in ^{this} med. for 3 days.

Freeze + thaw 3X — entire cells + med.

This is crude stock. Should be $1-5 \times 10^8$ pfu/ml

Store at -20° — Quite stable.

Plaque assay BSC-1

V Ag assay — Ab fr. Flow lab.

SU 40 labile at 37° — $1/2$ time of ≈ 2 days. (PV ≈ 7 days)

To conc. virus, pellet crude lysate 30,000 rpm
x 2 hrs + resuspend.

DOC 1/2% final
Trypsin .01% final

750 ml crude lysate

35000 rpm 2 1/2 hrs. - MSE

5 ml super left in each tube + pellet scraped into it (45 ml)

sonicated 3 min. Overnite 4°

Homog by hand to break up clumps

45 ml

+ 2.25 ml 0.25% trypsin

+ 2.25 ml 10% DOC (final 0.5%)

37° - 30'

serwall 10,000 rpm - 30'

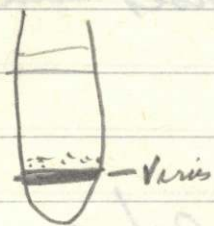
Super at 30,000 rpm 2 1/2 hrs.

Drained + 5 ml CsCl pH 7.9 $\rho = 1.304$

into each of 2 tubes 3' cent to clarify

Homog by hand + brought to 12 ml + CsCl sol'n

SW 39 32K 19 1/2 hrs



Hiyalag. 1x55c

Yield: 90 plates → 740 µg

Strain here is a) 777 - greater tend. to stick to cell debris

Other: b) 776 - virus release better.

c) Koch's str.

d) small pl mutant - Takemoto at NIH