

Polyoma Plaque Assay

11/9/67.

8 plates mouse embryo II⁻ cultures.

Sucked off medium and washed 2x in ~5 ml Tris.

Virus stocks.

LP 148 A 1.4×10^8 pfu/ml rec'd from MV.

Diluted as follows 10 ml \rightarrow 50 ml Tris-5% HS = 2.8×10^8 pfu/ml. (SAVED)

Made additional dilutions as follows. 10 ml \rightarrow 5.0 (1:500); 10 ml \rightarrow 4.0 (1:400) \rightarrow 0.5 \rightarrow 2.5 (280 pfu/ml)
 \rightarrow 0.1 \rightarrow 2.0 (70 pfu/ml)

- ① 0.2 ml of diln cty \sim ~~70~~ pfu/ml. (~14)
- ② " " " " " " " "
- ③ 0.2 ml of diln " \sim 280 pfu/ml. (~64)
- ④ " " " " " " " "

P16/S4K $\sim 5 \times 10^{10}$ pfu/ml.

Diluted as follows. 10 ml \rightarrow 5.0 ml Tris-5% HS = 10^8 pfu/ml (SAVED)

then

2.5×10^9 pfu/ml.
10 ml \rightarrow 4.0 (1:400); 10 ml \rightarrow 4.0 (1:400). 600 pfu \rightarrow 1:2 (300 pfu/ml)

\rightarrow 0.5 \rightarrow 2.5 (120 pfu/ml) \rightarrow 1:2 (60 pfu/ml)

- ⑤ 0.2 ml of diln cty 60 pfu/ml. (~12)
- ⑥ " " " " " " " "
- ⑦ 0.2 ml of diln cty 300 pfu/ml (~60)
- ⑧ " " " " " " " "

Absorbed for 30' at 37° then overlaid with agar-Eagles-3.5% HS.
Incubated at 37° for 2 days then shifted to 33°

Polyoma Plaque Assay.

On 11/20 (the 12th day) overlaid with neutral-red agar and put at 33°

On 11/22 (14th day) could see clear plaques on the LP 148 A infected plates but none the P16 plates.

On 11/24 (16th day) nice clear plaques on LP but no easily visible plaques on P16.

On 11/27 (19th day) counted plaques

LP 148 A

		Au	Predicted from titre of stock
①	9	} 12	~ 14
②	16		
③	36	} 33	~ 64
④	30		

Only few plaques seen with P16 ∴ left at 33°

On 11/30/67

		Expected on basis of titre
⑤	4	} ~ 12
⑥	lost	
⑦	6	} 60
⑧	4	

Either down by factor of 10 (stock is at 3⁴ per ml) or plating efficiency at 33° is lower.

Attempt to make plaques on PY-6

12/2/67

8 ~~plates~~ trays of 6 plates each. Plated at ca 2×10^5 cells/plate
on 11/30

Virus stock addition

LP148A 2×10^8 pfu/ml

10.0 \rightarrow 5.0 ml (1:50) \rightarrow 0.1 ml \rightarrow 5.0 ml (1:50) \rightarrow 0.6 ml \rightarrow 6.0 (1:10) 10^3 pfu/ml

Used 0.2 ml aliquots to infect

0.6 ml \rightarrow 6.0 (1:10) 10^2 pfu/ml

Plates washed in 25-6 ml Tris. Infected for 30' at 37° then
overlaid with agar and different media then incubated at 35° or 37°

(see next page)

The first two of each set infected with 2×10^8 pfu and the second
set infected with 2×10^7 pfu

38°

33°

* *
 (1) } 3.5% HTS
 (2) } + H. H. 42,
 (3) }
 (4) }

most cells dead - little
 staining - cells detached and
 floaty

* *
 (4) } 6.5% HTS
 (10) }
 (11) }
 (12) }

* *
 (17) } 0% HTS
 (18) }
 (19) }
 (20) }

(25) } 0% HTS
 (26) } 1:25
 (27) } Trypan
 (28) }

Well stained layer although
 much clumping: easily visible
 plaques: but only few/plate
 by 11 days

(33) } 0% HTS
 (34) } 1:100
 (35) } Trypan
 (36) }

Less well stained but
 clearly live cells in layer.
 lack of clear areas but not
 certain there are plaques

(41) } 0% HTS
 (42) } 1:250
 (43) } Trypan
 (44) }

Still less well stained
 but perhaps see plaques

(5) } 3.5% HTS
 (6) } + H. H. 42,
 (7) }
 (8) }

Same as 38°

(13) } 6.5% HTS
 (14) }
 (15) }
 (16) }

(21) } 0% HTS
 (22) }
 (23) }
 (24) }

(29) } 2% HTS
 (30) } 1:25
 (31) } Trypan
 (32) }

Same as 38°

(37) } 0% HTS
 (38) } 1:100
 (39) } Trypan
 (40) }

Same as 38°

(45) } 6% HTS
 (46) } 1:250
 (47) } Trypan
 (48) }

Same as 38°

* Stained on 12/7
 all H. H. stained on 12/9