



NIH AIDS Research & Reference Reagent Program

20301 Century Boulevard
Building 6, Suite 200
Germantown, MD 20874
USA

Phone: 240-686-4740
Fax: 301-515-4015
aidsreagent.org

HIV-1 Isolates from HLA-typed Individuals

Dr. Christian Brander, Nancy Brown
(Partners AIDS Research Center)

Virus Isolation Protocol:

Two round-bottom 5-mL tubes each containing 1×10^5 PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before and 200 μ L of plasma from the HIV-infected subject are spun for 3 hours at 37°C and 3000 rpm. The two tubes are transferred to a 25 cm² flask with 5 mL R20-50 and 5×10^6 PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before and incubated at 37°C and 5% CO₂ in a humidified incubator for 3 to 4 days. 10 mL R20-50 and 5×10^6 PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before are added and incubation is continued. At both one week and 1.5 weeks from infection, the culture is fed again with R20-50 by removing and replacing half of the supernatant volume, increasing total volume to 15 mL. Starting at 1.5 weeks after infection, viral growth is monitored by p24 ELISA. Two weeks after infection, and once a week subsequently, the culture is split by resuspending the cells, removing half the volume, and replacing with an equal volume of fresh R20-50 containing 5×10^6 PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before. At 2.5 weeks and weekly thereafter, culture is fed by removing half the volume without resuspending cells and replacing with an equal volume of R20-50. When the p24 indicates a sufficient concentration of virus, the culture is harvested by pelleting the cells at 1500 rpm for 10 minutes and removing the supernatant with a pipet.

R20-50 Medium:

RPMI 1640	500 mL
FBS (heat-inactivated 1 hr. at 56°C)	110 mL (<i>approximately</i>)
L-Glutamine (200 mM)	5 mL
Penicillin/Streptomycin	5 mL
<i>(Penicillin is 5000 I.U./mL, Streptomycin is 5000 μg/mL in the stock)</i>	
HEPES buffer (1 M stock)	6.2 mL
IL-2	280 μ L of 1×10^5 units/mL, 0.22 μ filter-sterilized
<i>(Medium is therefore approximately 50 units/mL)</i>	

HIV Isolates (Peru)			
<i>(Virus isolated from plasma)</i>			
Cat No.	Reagent Name (Code) Lot No.	p24/mL (pg)	TCID 50/mL
11635	222: H-2003-003 12/16/03	157,736	3.7E+05
11636	231: H-2004-012 2/12/04	191,849	5.6E+05
11637	233: H-2004-022 3/16/04	138,578	2.9E+05
11638	236: H-2004-025 3/23/04	161,247	3.8E+05
11639	237: H-2004-026 3/30/04	162,597	3.9E+05
11640	240: H-2004-029 3/30/04	103,040	1.9E+05
11641	H-2004-030 3/30/04	34,901	8.2E+04
11642	H-2004-031 3/31/04	77,285	1.4E+05
11643	244: H-2004-033 1/31/04	61,016	1.1E+05
11644	H-2004-035 4/6/04	81,321	1.4E+05
11645	251: H-2004-040 4/14/04	137,501	2.9E+05
11646	256: H-2004-057 8/10/04	81,712	1.5E+05
11647	259: H-2004-061 8/17/04	137,716	2.9E+05
11648	H-2004-062 8/16/04	54,537	1.0E+05
11649	265: H-2004-068 8/25/04	104,114	1.9E+05
11650	270: H-2004-071 9/7/04	12,047	6.2E+04
11651	H-2004-079 10/11/04	45,613	9.4E+04
11652	279: H-2004-080 10/11/04	129,223	2.6E+05
11653	H-2004-086 11/15/04	77,456	1.4E+05
11654	285: H-2004-089 1/25/05	85,678	1.5E+05