Centre for AIDS Reagents

ARP956

PCR Reference Kit / 95 Series

Datasheet.

CAUTION

THESE REAGENTS ARE NOT FOR ADMINISTRATION TO HUMANS. AS WITH ALL MATERIAL OF BIOLOGICAL ORIGIN THESE SAMPLES SHOULD BE REGARDED AS POTENTIALLY HAZARDOUS TO HEALTH. THEY SHOULD BE HANDLED IN ACDP (ADVISORY COMMITTEE ON DANGEROUS PATHOGENS) CLASS 2 LABORATORY.

PLEASE READ THE ENCLOSED DOCUMENTS CAREFULLY.

ON RECEIPT, PLEASE STORE SAMPLES AT -70°C.

PLEASE NOTE: Only coded DNA template samples are included. This set consists of 10 samples. For further information please refer to the publications.

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The Reference Reagents/95 Series.

The reference reagents comprise 10 tubes and have previously been tested in two International Collaborative Studies (Bootman JS and Kitchin PA, 1992; JS and Kitchin PA, 1994).

In house testing of the 95 series DNA templates gave the results as shown in table 1:

TABLE 1 DNA Templates

No. copies / 2.5µl	No. assays +ve / total no. or assays	% assays +ve
0 (H_2O)	0/21	0%
0 (Carrier DNA)	0/35	0%
0.1	3/27	11%
1.0	21/36	58%
10	29/29	100%
100	17/17	100%
1000	12/12	100%
10,000	12/12	100%

Reagents required for reactions:

Mineral Oil,
H_2O,
10 x buffer,
$MgCl_2$ (50mM),
DNTP mix (1.25mM each),
DNA polymerase,
Primers (4µM), gag, pol and env. – See references for details,
Coded DNA samples.

Coded DNA Samples / 95 Series.

Sample	Code	No of Tubes	Vol / Tube	Total Vol	Max No. of Assays
A	A/95	1	100µl	100µl	40
B	B/95	1	50µl	50µl	20
C	C/95	1	50µl	50µl	20
D	D/95	1	50µl	50µl	20
E	E/95	1	50µl	50µl	20
F	F/95	1	50µl	50µl	20
G	G/95	1	100µl	100µl	40
H	H/95	1	50µl	50µl	20
I	I/95	1	50µl	50µl	20
J	J/95	1	100µl	100µl	40

The coded DNA samples include linearised DNA from a plasmid containing a full length HIV genome, but completely lacking the 5' LTR and 150 bases of the 3' LTR. It is diluted in human carrier DNA known to be negative for HIV and Hepatitis B (HBsAg).

Use of the reagents.

Provided reagents should be stored at -20°C.
Before opening the tubes centrifuge briefly to collect the contents at the bottom.

Acknowledgements.

Publications should acknowledge the donor of the reagent and the Programme EVA Centre for AIDS Reagents. Suggested wording can be found on our website at http://www.nibsc.ac.uk/spotlight/aidsreagent/index.html in the "Acknowledgements" section.
Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR (this can be electronically or as a paper copy)

References:

HIV-1 Primers
Simmonds P et al, (1990), J Virol **64**: 864-872;

Collaborative Study 1
Bootman JS et al, (1992), J Virol Meth **37**: 23-42;

Collaborative Study 2
Bootman JS et al, (1994), J Virol Meth **49**: 1-8.

Protocol (NIBSC/MRC collaborative study – Autumn 1990)

Always centrifuge tubes briefly before opening.

- Label tubes 1-10 for gag assays, 11-20 for pol assays and 21-30 for env assays.
- Aliquot 50µl mineral oil into each assay tube.
- Prepare a master mix for each primer pair. Each master mix is sufficient for 10 tubes plus one extra.

"Gag"-Tubes 1-10

10 x Buffer	55.0µl
50mM $MgCl_2$	13.2µl
dNTP mix	88.0µl
H_2O	308.55µl
Primer HG1214N	27.5µl
Primer HG1686C	27.5µl
Taq Polymerase	2.75µl
Total	522.5µl

Mix gently and centrifuge briefly.

"Pol"-Tubes 11-20

10 x Buffer	55.0µl
50mM $MgCl_2$	11.0µl
dNTP mix	88.0µl
H_2O	310.75µl
Primer HP4149N	27.5µl
Primer HP4392C	27.5µl
Taq Polymerase	2.75µl
Total	522.5µl

Mix gently and centrifuge briefly.

"Env"-Tubes 21-30

10 x Buffer	55.0µl
50mM $MgCl_2$	15.4µl
dNTP mix	88.0µl
H_2O	306.35µl
Primer HE6539N	27.5µl
Primer HE6976C	27.5µl
Taq Polymerase	2.75µl
Total	522.5µl

Mix gently and centrifuge briefly.

(The $MgCl_2$ has been optimised for each pair of primers).

- Aliquot 47.5µl of "*gag*" master mix into assay tubes 1-10.
- Aliquot 47.5µl of "*pol*" master mix into assay tubes 11-20.
- Aliquot 47.5µl of "*env*" master mix into assay tubes 21-30.

- Add 2.5µl of template (coded samples A-J).

Ie. Add samples in order A-J to tubes 1-10 for *gag* assays, 11-20 for *pol* assays and 21-30 for *env* assays.

- Microfuge tubes briefly to mix.

- Amplification:

Cycle 1	1.5 mins at 94°C, 2.0 mins at 55°C, 3.0 Mins at 72°C.
Cycle 2-39	1.0 mins at 94°C, 2.0 mins at 55°C, 3.0 mins at 72°C.
Cycle 40	1.0 mins at 94°C, 2.0 mins at 55°C, 10.0 mins at 72°C, 4°C hold.

- Post PCR Analyses.

Analyse 10µl (ie. 1/5 of reaction volume) of post PCR material on a 1.75% agarose gel. Stain with Ethidium Bromide.

Southern Blot and hybridise using *gag*, *pol* and *env* specific probes as appropriate.

Appendix

Assay Components		**Final Concentration in Assay**
PCR Buffer		50mM KCl 10mM Tris pH8.3 0.01% gelatin
$MgCl_2$ gag	*Gag* assays	1.2mM
	Pol assays	1.0mM
	Env assays	1.4mM
DNTPs		200μM
Primers		0.2μM
Taq Polymerase		1.25 units

HIV-1 Primers

HG1214N	5'- GGT ACA TCA GGC CAT ATC ACC
HG1686C	5'- ACC GGT CTA CAT AGT CTC
HP4149N	5'- CAT GGG TAC CAG CAC ACA AAG G
HP4392C	5'- TCT ACT TGT CCA TGC ATG GCT TC
HE6539N	5'- GAG GAT ATA ATC AGT TTA TGG
HE6976C	5'- AAT TCC ATG TGT ACA TTG TAC TG

Primers are named on the following basis (eg HG1214N):

H	**H**IV.
G, P, E	***G****ag*, ***P****ol*, ***E****nv*.
1214	Coordinate of the 5' base of the primer, taken from the Los Alamos database 1989 entry for HIV HXB2.
N	**N**ormal strand.
C	**C**omplementary strand.

The primers will amplify target sequences to give products of the following sizes:

Gag	473 base pairs
Pol	244 base pairs
Env	438 base pairs

Please note: Doublet bands may be observed under certain assay and analysis conditions.

Coded DNA Samples

Sample	Template / 2.5μl
A/95	Carrier DNA, negative for HIV-1
B/95	1000 molecules of HIV-1 DNA
C/95	0.1 molecules of HIV-1 DNA
D/95	1.0 molecules of HIV-1 DNA
E/95	10 Molecules of HIV-1 DNA
F/95	100 molecules of HIV-1 DNA
G/95	H_2O control
H/95	1000 molecules of HIV-1 DNA (duplicate of B)
I/95	10,000 molecules of HIV-1 DNA
J/95	H_2O control

Samples B, C, D, E, F, H, I were diluted in human carrier DNA (40μg/ml) known to be negative for HIV-1.