

Viral integration detection Kit

Contents of the kit (for 10 or 30 samples):

1. Red cell lysis buffer (for blood samples)
2. Dissociation buffer
3. Linear DNA precipitation reagent
4. Buffer for DNA solubilization
5. Primer set for Actin
6. Primer set for HHV-6 or EBV or HHV-7
7. Protocol booklet

Schematic representation of the Viral integration Kit

Cells (1×10^4 to 1×10^6) or blood (50 to 100 μ l)

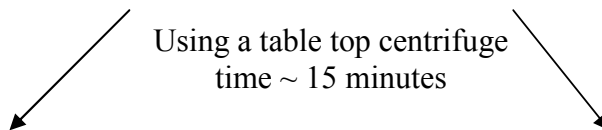


Add dissociation buffer



Incubate for 10 minutes

Separate into integrated and episomal DNA fractions by centrifugation



Chromosomal DNA (containing integrated viral genome)

Low molecular DNA (containing episomal, linear viral DNA)



Run QC-PCR

Primers

HHV-6 or other viral primers
Actin primers



Run QC-PCR

Primers

HHV-6 or other viral primers
Actin primers

PCR Results:

Actin is only detected in the integrated fraction. Actin is not detected in the low molecular DNA fraction (or detected in less than 1/100 ratio compared with integrated fraction see explanation below)

HHV-6 or other viruses will be detected in the chromosomal fraction if it is integrated otherwise it will be detected in the low molecular fraction episomal or linear.

If it is detected in both fractions, it is present in both forms. Using QC-PCR the ratio between the two forms can be quantitated.

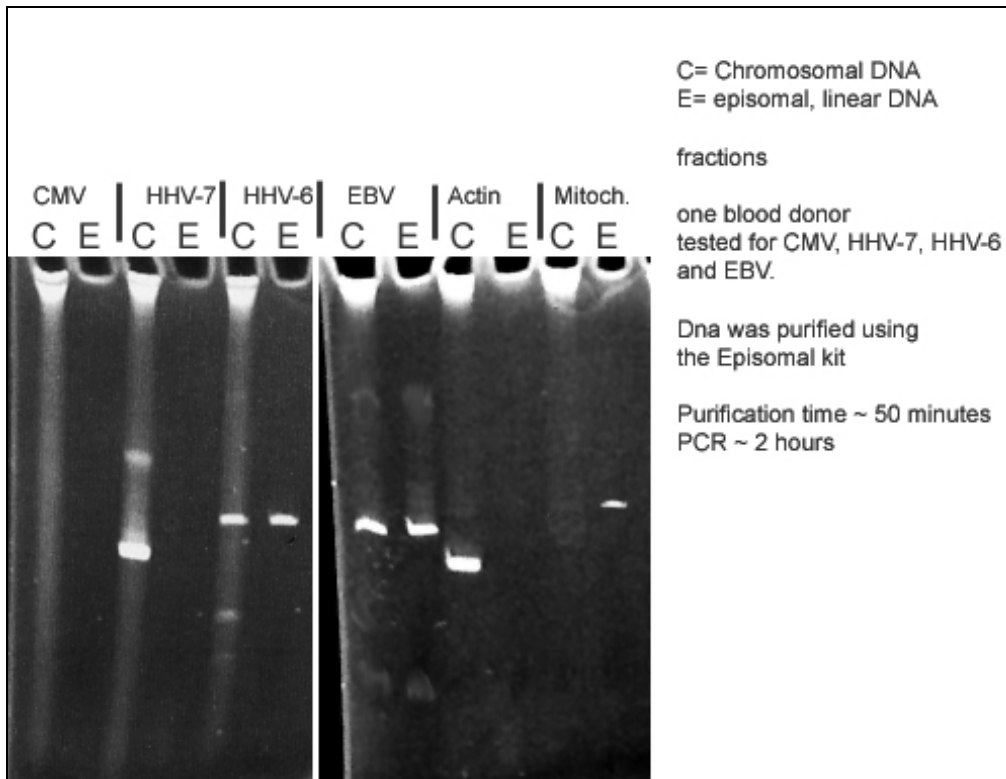
The control primer (Actin) is indicating that the assay was working for separation of free DNA from the integrated DNA. Small amount of Actin can be found in the episomal, linear DNA fraction because in any cell preparation it is always some dead cells are present which releases degraded cellular DNA. However, generally it should be less than 1% of the total DNA, therefore in a quantitative or semi-quantitative PCR the ratio between cellular actin and degraded actin should be 100 to 1 or better.

It is important to use QC-PCR or semi-quantitative PCR. Endpoint PCR 35 to 40 cycles will amplify low level of degraded actin to saturation therefore on gel analysis the quantitative difference between the two fractions will not be detected or will be very close.

As controls for EBV Namalva (ATCC) and Raji (ATCC) cell lines can be used. Namalva has 2 copies integrated EBV genome while Raji has 10 copies integrated 30 copies episomal EBV genome per cell.

For HHV-6 we use the HSB-ML (Bioworld Consulting Laboratories) cell line which has 8 copies integrated HHV-6 genome and HSB-F (Bioworld Consulting Laboratories) which has 20 copies integrated and ~30 copies linear HHV-6 genome per cell.

Picture of the results from a blood donor using regular PCR (30 cycles) and the product analyzed by gel electrophoresis



The result is indicating that CMV is not present; HHV-7 only present in the integrated form; HHV-6 present both in integrated and linear form; EBV present in both integrated and episomal form.

Control Actin is only present in the integrated form; Mitochondrial DNA only present in the free episomal form.