Induction of HHV-6 and HHV-7 in latently infected cell lines

Cell line	viral genome
HSB-ML	HHV-6
HSB-F	HHV-6
HSB-M	HHV-6
END-HHV-6	HHV-6
Lam/BH	HHV-6/EBV
ST-7	HHV-7

To induce the virus replication in the inducible cell lines it is very important that the cell lines be in stationary phase.
The best and cheapest method is, to let the cells grow to high density in the cell culture medium (~ 1.5-2.0 x 10^6 cells/ml). Generally, it is achieved 3 to 4 days after adding fresh medium to the cells.

Medium: RPMI 1640 supplemented with 10% FCS

<u>Subculturing of the cells</u>

1. After taking out the cells from the liquid nitrogen storage, grow the cells for at least one week before attending to induce viral replication.
2. When splitting the cell culture, dilute the cell culture with fresh medium to ~ 2-3 x10^5 cells/ml.
3. When the cells are between 1.5-2.0 x 10^6 cells/ml (3-4 days) split them again.

<u>Induction of the cells</u>

1. Pellet the cells which are in stationary phase (~1.5-2.0 x 10^6 cells/ml) by centrifugation (~1,000 rpm for 10 minutes).
2. Resuspend the cell pellet in RPMI 1640 medium with 5% FCS to a cell concentration of ~ 1.5-2 x 10^5 cells/ml and add the chemicals for induction (see composition bellow).
3. Incubate the cells at 37°C for 6-10 days.
4. Check induction by immunofluorescence assay using monoclonal antibodies to EA and late antigens at day 3, 6 and 9.

<u>Composition of inducers for the cell lines</u>

Every cell line responds differently to each inducer the best is to test each method in small scale and use the best inducer for large scale induction.

Method 1: TPA 20ng/ml plus hydrocortisone 5x 10^{-5} M
Method 2: TPA 20ng/ml plus 3 mM Nabutyrate
Method 3: TPA 20 ng/ml plus trichostatin A (TSA) 80 ng/ml