Note from Phillip West, PhD: This protocol is adapted from the Prusty protocol on the HHV-6 website. The biggest leap forward for us was using a water bath based sonicator as opposed to a probe sonicator. Postdoctoral researcher Christine Birdwell is continuing to modify this protocol, but the latest batch of HHV-6B was pretty high-titer based on the number of positive cells we observed between 3-7 days of culture. HHV-6A GS has been a little more tricky, but we think we are still able to see ~30% of cells coming up positive from our prep. One thing we have noticed is that if the uninfected Molt or HSB2 cells remain in culture for a long time, their ability to yield high titer stocks decreases. The ability to infect with a cell-free HHV6 viral prep also seems to decrease. We thaw from the original stocks every 30 days to minimize this. Perhaps this is related to the viral genome instability. Give this, cord blood culture may be the best for generating high titer stocks, and we should periodically re-seed the immortalized lines with cord blood derived viruses to minimize drift.

West Lab HHV-6 cell free virus stock protocol

Day 1

1. Per T^125 flask, mix 1x10^7 uninfected MOLT-3 cells with 10^6 HHV-6 infected cells in a small volume (~5mL) and incubate in a cell incubator at 37°C in a tilted position. Five flasks are done at a time.

2. After 2hr incubation, add fresh media to 20-25mL total and return flasks to the incubator (no tilt).

Day 4-5

- Cells should be collected when ~90% of cells are enlarged and show cytopathic effects, but before the majority have lysed by light microscope observation.

- Ultracentrifuge tubes (Beckman Ultra Clear 1x3^1/2 in [25x89mm] #344058) should be cleaned prior to use (10 minutes in 10% bleach followed by 2hrs in 70% Ethanol before drying in the biological safety cabinet where they will be loaded).

- Prepare ice cold water and a bucket of ice in advance of collection.

3. Collect cells in 50mL conical tubes (~30mLs per tube) and keep on ice.
4. Lyse infected cells in a water bath sonicator (Cole-Parmer Ultrasonic cleaner 8895-52 [60 Degas/minute]) using ice cold water in 2 minute intervals, resting cells on ice in between. After every 2 minute sonication, count trypan blue negative cells. Sonication should continue until ~less than $1 \times 10^5$ intact cells remain (4-6 minutes). Do not sonicate more than 10 minutes total. Make sure the water in the bath sonicator remains cold, change as needed.

5. Spin down lysed cells with culture fluid at 3,500 rpm for 30min at 4°C.

6. Filter supernatant through a 0.45-µm filter (Nalgene Rapid Flow 168-0045), which contains viral particles. Load filtered supernatant into ultracentrifuge tubes (~35mL per tube), using a scale to ensure they are evenly balanced. Use tissue culture sterile PBS if needed for extra volume to balance.

7. Pellet virus by ultracentrifugation at 13,500 rpm for 3hr at 4°C (Surespin 360 rotor in a Sorvall WX Ultra 80 centrifuge).

8. Carefully remove media and re-suspend the virus pellet in ~500µL of complete media (10%FBS RPMI).

9. Incubate virus suspension overnight on ice in 4°C.

10. Aliquot virus in 10-15µL portions and flash freeze on dry ice or liquid nitrogen before storing at -80°C.

11. The HHV-6 titer expressed as the 50% tissue culture infective dose (TCID$_{50}$) should be determined by infecting fresh MOLT-3 cells at different dilutions and immunostaining the infected cells using the HHV-6 p41 (C-5) antibody (HHV-6 Foundation) [1:400 dilution used]. Viral titer and TCID$_{50}$ value is calculated using Reed-Münch formula.

Using this protocol, a 1:1000 dilution (5ul into 5ml culture) results in roughly 50% of cells being p41 positive by day 7.